ETIOLOGICAL EVALUATION OF IDIOPATHIC GRANULOMATOUS MASTITIS BY A PROSPECTIVE CASE CONTROL STUDY

A dissertation submitted in partial fulfillment of M.S General Surgery Branch I

Examination of the Tamil Nadu Dr. M.G.R University, Chennai to be held in 2018

CERTIFICATE

This is to certify that the dissertation titled "Etiological evaluation of Idiopathic Granulomatous Mastitis by a prospective case control study" is a bonafide work of Dr. Mithun Raam, carried out under our guidance towards partial fulfillment of M.S General Surgery Branch I Examination of the Tamil Nadu Dr. M.G.R University, Chennai to be held in 2018.

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DECLARATION CERTIFICATE

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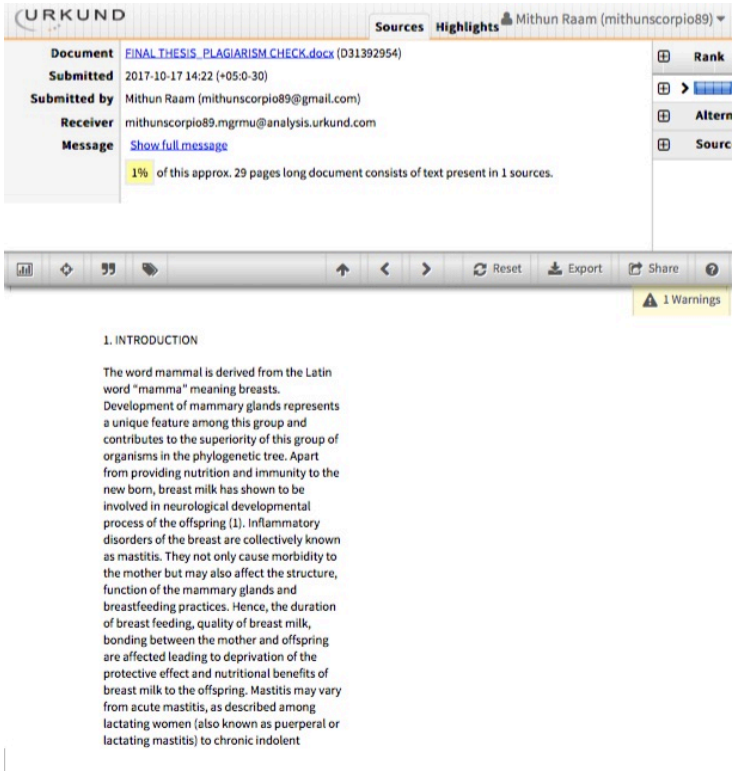

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ACKNOWLEDGEMENTS

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I would also like to acknowledge the support from the Departments of General Surgery and Endocrine Surgery and the ward staff of the General Surgery and Endocrine Surgery wards for the help in participation and conduct of the study.

I am grateful to my parents Mr. K. Raamamoorthy and Mrs. Rani Raamamoorthy, my brother Er. Gokul Ramamurthy for lending support and words of encouragement in times of difficulties without which this dissertation could not have been completed.

Last but not the least; I would like to thank the participants for their patience and contribution, despite all the odds.

IRB APPROVAL

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Etiological evaluation of idiopathic granulomatous mastitis by a prospective case control study.
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Dear Dr. Mithun Raam,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Etiological evaluation of idiopathic granulomatous mastitis by a prospective case control study" on October 20th 2015.

I enclose the following documents:-
1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. NIHAL THOMAS
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The Committee reviewed the following documents:

1. IRB Application format
2. Patient Information Sheet and Informed Consent Form (English, Tamil, Telugu, Hindi, Bengali)
3. Questionnaire
4. Cvs of Drs. B.Antonisamy , Marie Therese Manipadam, Vijay Abraham, Mrs. Gracy Varghese, John A Jude, John Mathew, Joy Sarojini Michael, Jayaprakash Muliyil, M.J. Paul
5. No. of documents 1 - 4

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on October 20th 2015 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

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Dr. RatnaPrabha	MBBS, MD	Associate Professor, Clinical Pharmacology, CMC, Vellore.	Internal, Pharmacologist

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Etiological evaluation of idiopathic granulomatous mastitis by a prospective case control study" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in)

Fluid Grant Allocation:

A sum of 1,00,000/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 50,000/- INR (Rupees Fifty Thousand only) each will be released at the end of the first year as 2 nd Installment

Yours sincerely

Dr. Nihal Thomas
Secretary (Ethics Committee)
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TABLE OF CONTENTS

1. INTRODUCTION

The word mammal is derived from the Latin word “mamma” meaning breasts. Development of mammary glands represents a unique feature among this group and contributes to the superiority of this group of organisms in the phylogenetic tree. Apart from providing nutrition and immunity to the new born, breast milk has shown to be involved in neurological developmental process of the offspring (1).

Inflammatory disorders of the breast are collectively known as mastitis. They not only cause morbidity to the mother but may also affect the structure, function of the mammary glands and breastfeeding practices. Hence, the duration of breast feeding, quality of breast milk, bonding between the mother and offspring are affected leading to deprivation of the protective effect and nutritional benefits of breast milk to the offspring.

Mastitis may vary from acute mastitis, as described among lactating women (also known as puerperal or lactating mastitis) to chronic indolent mastitis such as Granulomatous mastitis. The former condition which is more common, is easily treatable and has a lesser propensity for recurrence and prolonged morbidity. The latter however tends to follow an indolent protracted clinical course resulting in significant morbidity.

Granulomatous Mastitis is a chronic inflammatory condition affecting the mammary tissue which was first described in 1972 in Israel as a lesion mimicking breast carcinoma (2). Since its first description, there have been a number of isolated case reports and case series describing this entity from different parts of the world. Though it is a benign disease, unlike other forms of

mastitis, it has been found to have an indolent course. Patients most often present with multiple recurrences and relapses causing significant medical morbidity and psychological turmoil.

Granulomatous mastitis presents as a wide clinical spectrum. In some patients, it presents only as a palpable breast lump which may be clinically indistinguishable from a malignant breast lump. On the other hand, it may present with overt signs of inflammation, as in other types of mastitis such as redness, warmth, tenderness, skin ulceration or multiple sinuses discharging pus.

Thus, Granulomatous mastitis presents a diagnostic as well as therapeutic challenge to the treating physician. Furthermore, it continues to be an ill-understood disease. Initially described as a rare disease entity, the number of reported cases and published literature are on the upward trend which may probably due to under-diagnosis and underreporting of this condition.

Most of the current literature on Granulomatous mastitis describes its clinical spectrum, diagnosis and management. There are very few studies that have been published about its etiopathogenesis resulting in an inadequate understanding of the disease and thereby a variety of theories of causation and therapeutic strategies. In a country like India, where infectious diseases are common, these patients are treated with prolonged antibiotic courses and empirical Anti-tubercular therapy based on the granulomatous histopathology, with no microbiological evidence of infection with Mycobacterium species.

Hence, an understanding of the causative factors and elucidation of the pathogenesis would help in defining the diagnostic criteria and formulation of better targeted therapeutic strategies and preventive measures to reduce the burden of this disease and its associated morbidity.

The increasing disease burden, the associated morbidity of this disease both to the mother and child and paucity of literature, especially from Asia and the Indian subcontinent led the investigators of this study to embark on this study. This study aims to analyze etiological factors which may be demographic, patient-related or environment-related and to test carefully for infection and autoimmunity that may play a role in causation of granulomatous mastitis.

2. AIMS AND OBJECTIVES

AIM: To evaluate the possible etiopathology of Idiopathic Granulomatous Mastitis (IGM)

PRIMARY OBJECTIVES:

1. To test for uncommon infections in the breast as the cause of IGM by routine bacterial smear and culture, AFB smear and culture for typical and atypical mycobacteria and special tests for fungal etiology.

2. To assess serum markers for autoimmunity (ANA, specific autoantibodies, Immunoglobulin levels) in patients with IGM.

SECONDARY OBJECTIVES:

1. To analyse demographics and clinical profile of patients with IGM.

2. To analyse individual hormonal profile and breastfeeding practices as possible risk factors in IGM.

STUDY HYPOTHESIS: Idiopathic granulomatous mastitis is a chronic inflammatory disorder of the breast that is mediated by an uncommon infection or autoimmune phenomenon.

3. REVIEW OF LITERATURE

3.1 STRUCTURE AND FUNCTION OF MAMMARY GLANDS

3.1.1 EMBRYOGENESIS OF MAMMARY GLANDS

Mammary glands are essentially derived from modified, differentiated sweat glands. Around 4th to 6th week of gestation, mammary progenitor cells arise with proliferation of epithelial cells in the thorax. This, results in the formation of two ridges extending from the base of the upper limb bud, in the axilla to the base of the lower limb bud, in the inguinal region called the mammary ridges or milk lines. Majority of the mammary ridge eventually atrophies, except for a cluster of cells in the 4th intercostal space in the pectoral region which give rise to the primary mammary bud (Fig 3.1A and 3.1B) (3).

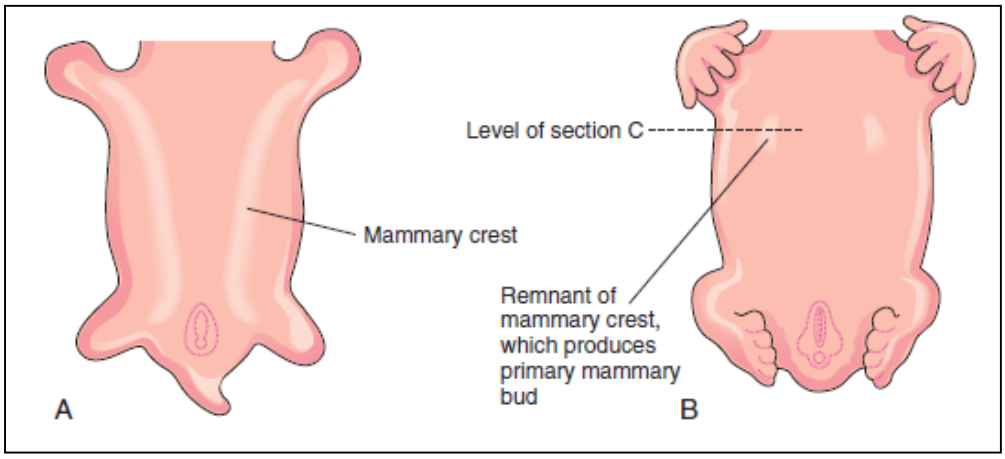

Fig 3.1 A and B: Embryonic development of breasts

By the end of the first trimester, the primary mammary buds grow into the mesenchymal tissue, under the stimulation derived from mesenchymal regulatory factors (Fig 3.1C and 3.1D). Secondary mammary buds arise along the basolateral margin and continue to invaginate into the underlying mesenchyme to get surrounded by a zone of fibroblastic cells which differentiate to form fibroblasts, smooth muscle cells, capillary endothelial cells, and adipocytes (Fig 3.1E) (3).

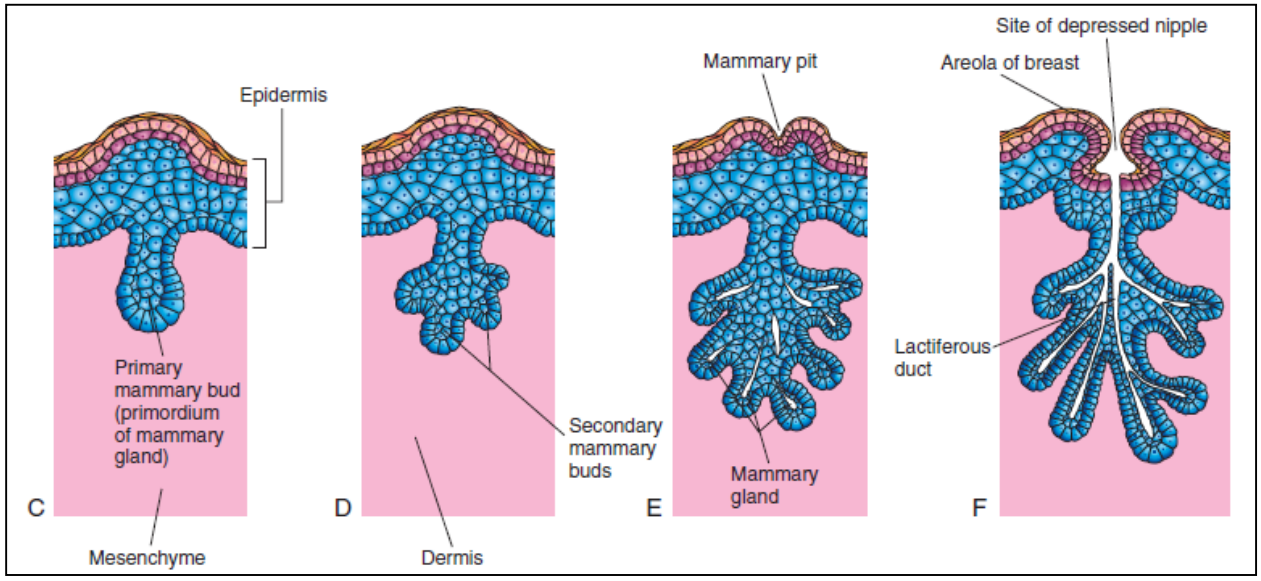

Fig 3.1 C-F: Embryonic development of breasts

Branching and canalization of the secondary mammary buds result in the formation of lactiferous ducts. The epidermis forms a depression, known as the mammary pit during the third trimester which will give rise to the nipple-areolar complex (Fig 3.1F). Lactiferous ducts drain into retroareolar ampullae that coalesce at the mammary pit onto the skin. At the end of embryonic development, the term infant develops 15-20 lobes with a corresponding lactiferous duct opening out onto the overlying skin.

3.1.2 DEVELOPMENT OF MAMMARY GLANDS

In the postnatal period, the infant breast shows features of both morphological and functional development which are signified by differentiation of glandular structures and development of the secretory epithelium respectively. The extent of development varies in the infant breast and has been elaborated by Anbazhagan et al (4). These changes may occur in varying proportions and does not follow a linear progression. The development of the mammary glands then enters a quiescent phase from 2 years of age till puberty.

3.1.2.1 CHANGES IN PUBERTY

At puberty, the breast undergoes modification both at morphological and ultra-structural levels which is influenced by various hormonal factors. Increasing secretion of follicle stimulating hormone and luteinizing hormone occurs under the stimulation from the hypothalamus. The increasing levels of hormones causes activation of primordial ovarian follicles and secretion of oestrogen. Oestrogen induces ductal development and branching apart from development of the connective tissue and vascularity. Lobular development takes place in the presence of increasing progesterone levels in the luteal phase of the menstrual cycle. Four different types of lobular development have been described. Type I lobules have a short terminal duct with a cluster of

surrounding secretory cells called alveoli. Type II, III and IV lobules have a terminal duct branching into several ductules and an increasing number of alveoli. The nulliparous breast contains mainly type 1 lobules whereas the breast of women who have gone through pregnancy

and lactation has mostly type 4 lobules. The mammary glands stay inactive till pregnancy. Pregnancy brings about the next major change in the hormonal milieu.

3.1.2.2 DEVELOPMENT AT THE CELLULAR LEVEL

In the initial phase, there is increase in fibrous and fatty tissue within the stroma followed by elongation and branching of the ductal system. The epithelial layer develops into two layers, consisting of outer myoepithelial layer and inner luminal layer that can be further divided into ductal cells, lining the inside of the ducts, and alveolar cells, which secrete milk during lactation.

Ductal elongation and branching results in formation of multiple segmental and sub-segmental ducts which arise from the primary bud. These sub-segmental ducts eventually branch to form terminal ducts and terminal ductules or acini. Group of acini along with surrounding interlobular stroma forms the Terminal duct lobular unit (TDLU) which is the functional unit of breast (5). Many other hormones such as insulin, prolactin, corticosteroids and growth hormone play a minor role in the overall development of breast.

3.1.2.3 DEVELOPMENT AT THE MORPHOLOGICAL LEVEL

Morphological development of breasts in puberty begins at about 10 years of age. There is increase in breast tissue in the areolar region which gives rise to the primary breast mound (Fig 3.2A). Elevation of the breast tissue with increasing protuberance of the nipple occurs by 12 years of age (Fig 3.2B). Appearance of the secondary breast bud occurs by 14-15 years of age which is caused by accumulation of sub-areolar tissue (Fig 3.2C). The areolar tissue regresses in relation to the surrounding breast tissue giving rise to the adult contour of breast (Fig 3.2D). These changes have been described by Marshall and Tanner and have been formulated into a staging system known as Tanner's staging (6).

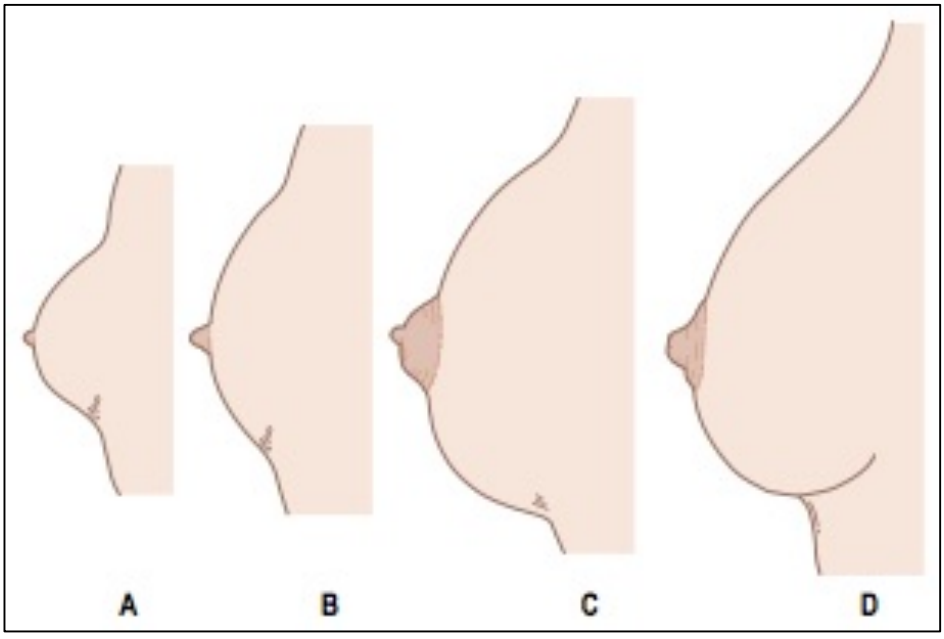

Fig 3.2 A-D: Morphological development of breasts at puberty

3.1.3 GROSS AND ULTRASTRUCTURAL ANATOMY OF BREAST IN THE ADULT

Mammary glands lie on both sides of the thoracic wall and extend from the second to sixth rib craniocaudally and from the lateral sternal border medially to the mid-axillary line laterally with extension into the axilla through the axillary tail of Spence. Breast tissue comprises varying admixture of both fatty and glandular tissue that depends on the age, breast volume, body mass and other factors. The nipple areolar complex is a hyperpigmented elevated area which serves as an outlet for milk production. The areola also has multiple modified sebaceous glands called Montgomery's tubercles that help in lubrication of the areola during suckling (Fig 3.3) (3).

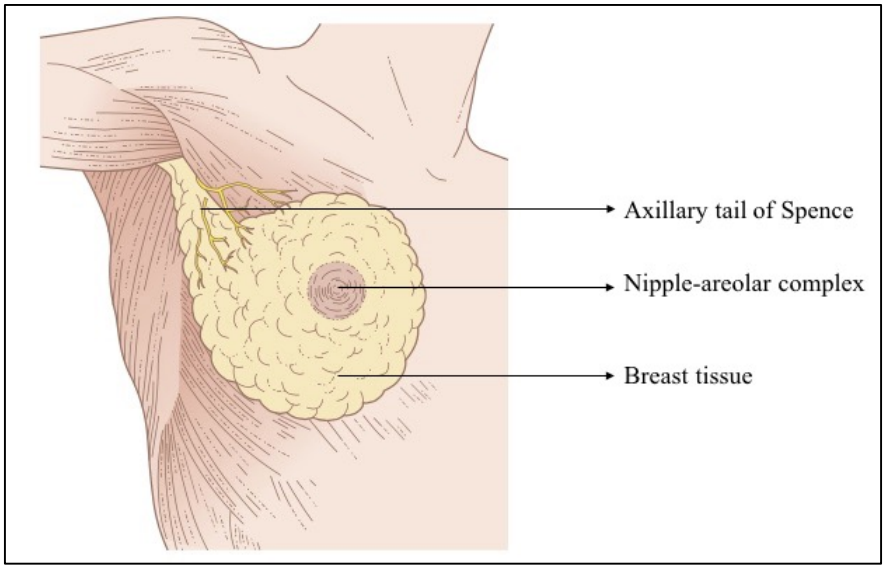

Fig 3.3 Gross anatomy of breast

The duct-lobular system of the breast comprises multiple lobes (15-20 in number) that are separated by fascia. Each lobe contains more than 40 lobules which in turn are comprised of almost 100 acini or alveoli. As described above, group of acini along with the surrounding

stroma forms the terminal duct lobular unit (TDLU) which is the basic secretory unit (Fig 3.4) (3). Eventually, secretions drain through lobar ducts into a lactiferous sinus which drains through the nipple.

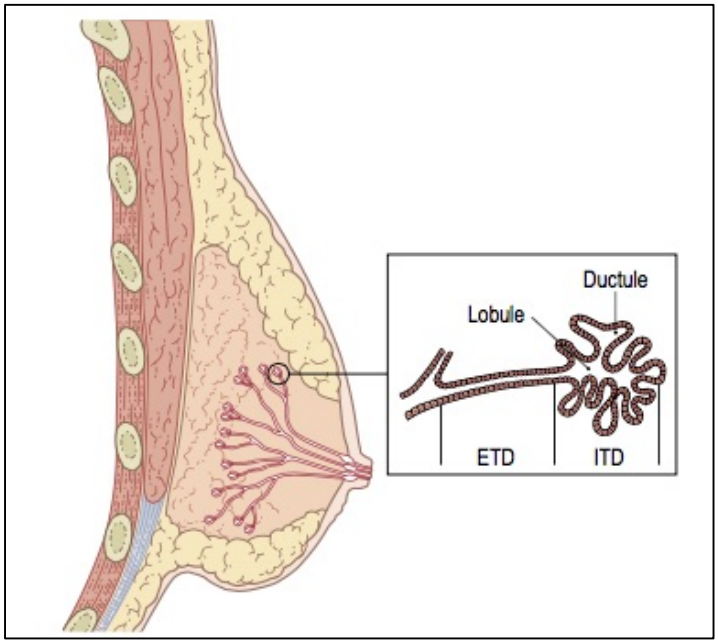

Fig 3.4 Ducto-lobular system (ETD- Extralobular terminal ductule; ITD – Intralobular terminal ductule)

3.1.4 CHANGES IN PREGNANCY AND LACTATION

During pregnancy, there is an increase in progesterone, placental sex steroids and other hormones such as placental lactogen and chorionic gonadotrophin which stimulates significant growth of lobulo-alveolar system. On microscopy, there is predominance of dilated alveoli and the two-layered epithelium changes to form a single layer within the alveolus.

The changes in the breast during pregnancy have been described below (3):

Week of gestation	Change
0	Resting breast ~ 200 gm in weight
1-4	Ductular sprouting/lobular formation
5-8	Breast enlarges/vascular engorgement/areolar pigmentation/predominant lobular formation
12	Large alveoli with single epithelial cell layer Beginning of colostrum formation
>20	Alveolar dilatation/colostrum formation/ New capillary formation/myoepithelial cell hypertrophy
Term	180% increase of mammary blood flow Weight approx. 400 g Fat droplet accumulation in alveolar cells

Table 3.1

Prolactin levels gradually rise to reach peak levels at term and subsequently fall, with rise once breastfeeding is initiated. Galactopoesis or production of milk occurs due to these increasing levels of prolactin and the absence of inhibitory effect of placental sex steroids soon after birth. Emptying of the breast helps in continuation of galactopoesis. Stasis of milk for more than 48 hours reduces milk secretion.

During lactation, the upper part of the alveolar cell breaks away and changes from columnar to cuboidal in shape. Lactose synthesis and milk protein synthesis occurs by stimulation of lactose synthetase and nuclear RNA polymerase under the influence of prolactin. Proteins and fats are excreted by apocrine secretion whereas lactose is excreted by aprocine secretion and inorganic ions by active and passive transport. Thus, milk secretion occurs across the luminal cell membrane ("blood-milk barrier"). Following this, re-secretion of milk proteins and cells occurs leading to elongation of the alveolar cell.

During the phase of weaning, involution starts in the breast. Reappearance of the characteristic two-layered alveolar epithelium occurs. There is increasing influx of histiocytes and lymphocytes that results in phagocytosis and apoptosis. The alveolar structures regress with preservation of the ductal structure. Despite this involution, there is some secretion that may be retained in the breast, even after lactation is stopped (7).

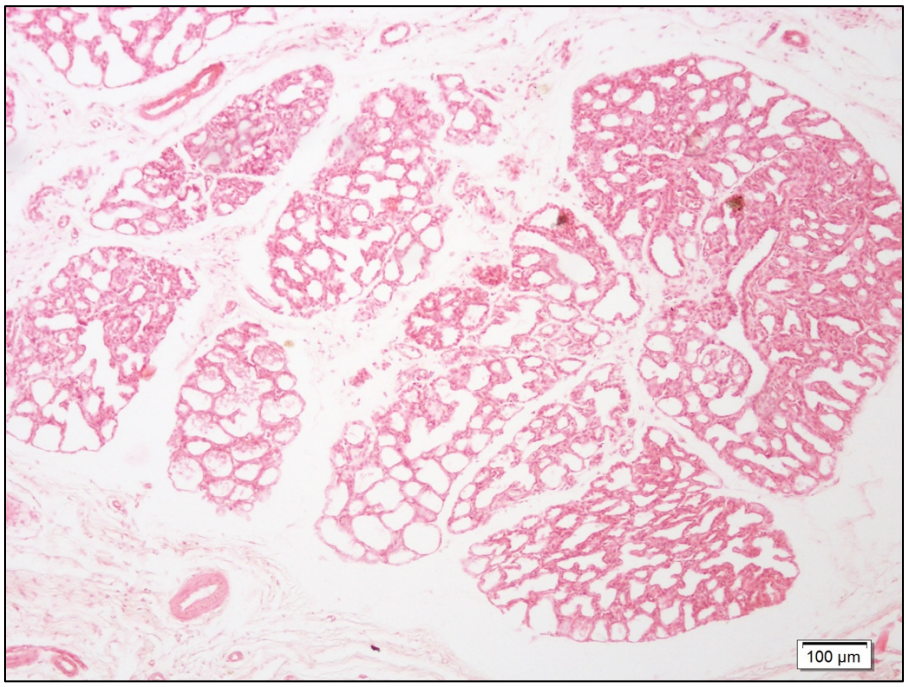

Fig 3.5 Histology of Lactating breast (Courtesy: Department of Anatomy, CMC, Vellore)

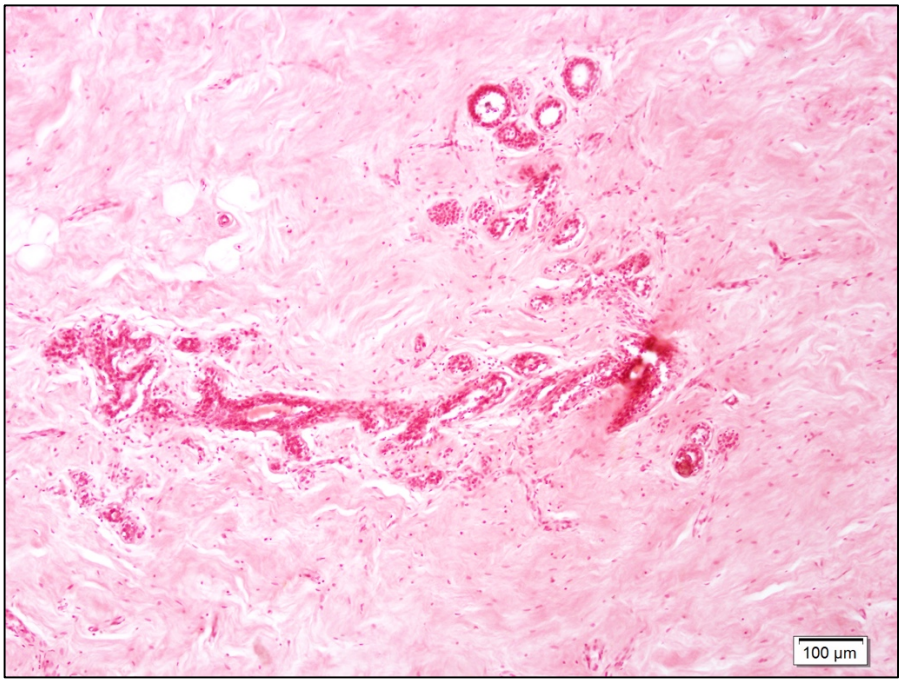

Fig 3.6 Histology of Non-lactating breast (Courtesy: Department of Anatomy, CMC, Vellore)

3.2. INFLAMMATORY DISORDERS OF THE BREAST

Mastitis denotes a group of inflammatory disorders involving the breast tissue. The overall incidence and prevalence of mastitis is largely underreported as the numbers quoted in literature are only women seeking medical attention and disease definitions vary in different studies. Inch and Von Xylander et al in a meta-analysis have shown the incidence of mastitis to range from 2% to 50% (8). In a prospective study done among 262 women from New Delhi, India in 2012, inflammatory disorders of the breast accounted for 10% of patients presenting to the out-patient clinic (9).

Mastitis may be classified in many ways. They may be classified according to temporal profile into Acute and Chronic or based on their etiopathogenesis. In a review article published by Kamal et al, Mastitis has been broadly classified into infectious, non-infectious and malignant based on their etiopathogenesis (Fig 3.4) (10).

The first group comprises simple mastitis (lactational or non-lactational), complex mastitis such as abscesses, infected cysts etc. and mastitis associated with specific diseases such as tuberculosis or fungal infections. Out of 197 patients, 67% of patients belonged to this group, mostly involving lactating women (37.9%) (10).

The second group comprises non-infectious conditions causing aseptic inflammation or chemical inflammation of the breast. This includes conditions such as periductal mastitis, idiopathic granulomatous mastitis, Plasma cell mastitis, diabetic mastopathy etc. and accounted for 27.4% of patients (10).

The third group comprises entities such as inflammatory breast carcinoma and malignant breast abscess which accounted for 5.6% (10) .

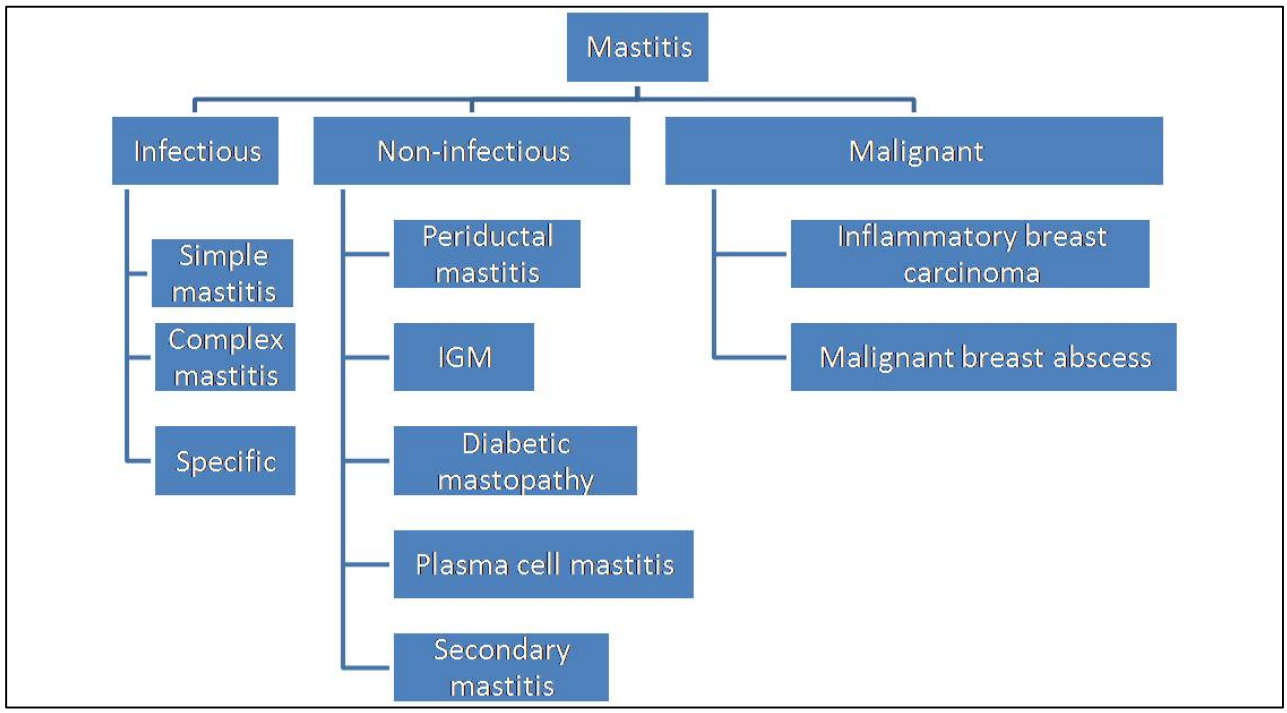

Fig 3.7 Classification of mastitis

This study focuses on Granulomatous mastitis and its etiopathogenesis. An understanding about this would be incomplete without an understanding of granulomatous inflammation in a broad perspective.

3.3. GRANULOMATOUS INFLAMMATION

Chronic inflammation, as opposed to acute inflammation persists for a long duration, sometimes lasting weeks or months. This is understood as an attempt to contain an offending agent that is difficult to eradicate resulting in a process where inflammation, tissue injury and attempts at repair occur simultaneously.

Granulomatous inflammation may be defined as a form of chronic inflammation which is characterized by macrophages, epithelioid cells and multinucleated giant cells which form a part of the mononuclear phagocyte system, along with lymphocytes, plasma cells and fibroblasts (11). They may be present in a diffuse manner or in the form of focal lesions called granulomas (Fig 3.10) (12). A detailed description of the different components of granulomatous inflammation is described below in an attempt to understand the pathophysiology.

3.4 COMPONENTS OF CHRONIC GRANULOMATOUS INFLAMMATION

3.4.1 MONONUCLEAR PHAGOCYTIC SYSTEM

The circulatory cells which are derived from the hematopoietic stem cells in the bone marrow and progenitor cells from the yolk sac and liver of the developing fetus are known as monocytes. These cells migrate into tissues and differentiate to form macrophages. The progenitor cells from the fetus develop into resident macrophages, such as microglia, Kupffer cells, alveolar macrophages and macrophages in the spleen and connective tissues where they lie in the dormant state. In the setting of inflammation, these monocytes migrate to areas, which is governed by

various chemotactic and activation mechanisms brought about by different chemical mediators and adhesion molecules (Fig 3.8) (12).

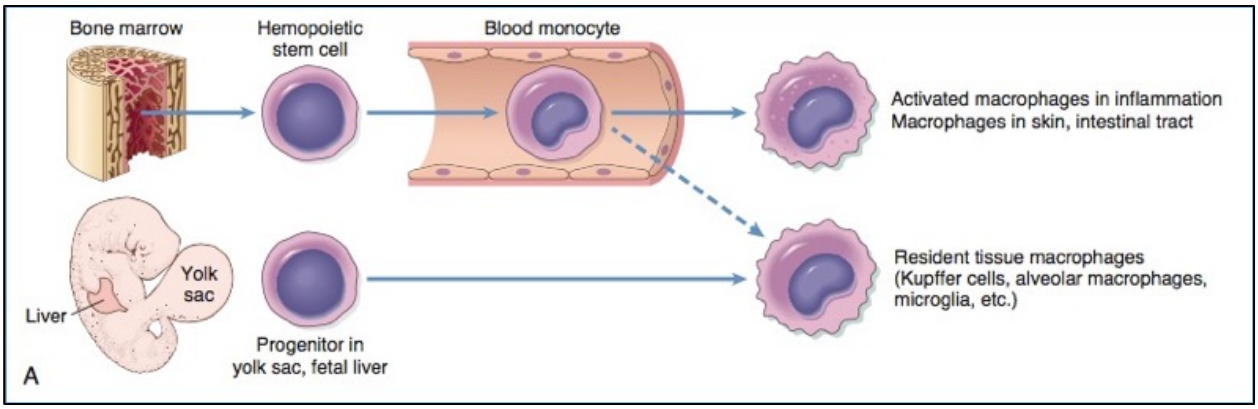

Fig 3.8 Mononuclear phagocytic system

Macrophages form the dominant cells in chronic inflammation. They ingest a wide variety of substances including microbes into membrane bound vacuoles by endocytosis. The macrophages then undergo activation followed by fusion of phagosome and lysosome allowing intracellular degradation and microbial killing. Microbial killing is done by superoxides, hydrogen peroxide and hydroxyl radicals and other microbicidal substances. The process is aided by the presence of antibodies. Digestion of particulate material and dead organisms is accomplished by lysosomes. Macrophage activation occurs by two different pathways resulting in different consequences (12).

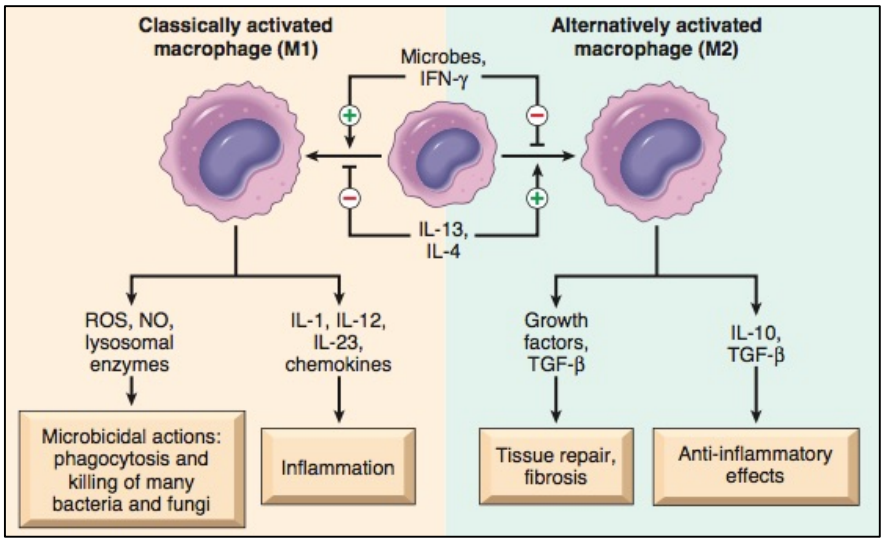

Fig 3.9 Pathways of macrophage activation

3.4.2 CLASSICAL PATHWAY

This pathway is brought about by microbial products such as endotoxins, by T cell–derived signals, importantly the cytokine IFN-γ in immune responses or by foreign substances including crystals and particulate matter. These cells also known as M1 macrophages, produce NO and ROS, upregulate lysosomal enzymes and secrete cytokines that stimulate inflammation.

3.4.3 ALTERNATIVE PATHWAY

This pathway is induced by cytokines other than IFN-γ, such as IL-4 and IL-13 which are produced by T lymphocytes and other cells. The macrophages activated by the alternative

pathway are principally involved in tissue repair and not in inflammatory response. They secrete growth factors that promote angiogenesis, activate fibroblasts, and stimulate collagen synthesis.

3.4.4 ROLE OF LYMPHOCYTES

Macrophages have complex interactions with lymphoid cells at different phases of the immune response. Antigen presentation to the T lymphocytes and B-lymphocytes initiates cell-mediated immunity and helps in amplifying and propagating the inflammatory process. These CD4+ T lymphocytes produce different types of cytokines that function differently(12):

- T_H1 cells produce the cytokine IFN-γ, which activates macrophages by the classical pathway.
- T_H2 cells secrete IL-4, IL-5, and IL-13, which recruit and activate eosinophils resulting in activation of the alternative pathway.
- T_H17 cells secrete IL-17 and other cytokines, which induce the secretion of chemokines responsible for recruiting neutrophils and monocytes

Lymphocytes and macrophages thus interact in a vicious cycle propagating chronic inflammation. Macrophages display antigens to T cells, express membrane molecules and produce cytokines that stimulate T-cell responses and activated T lymphocytes on the other hand produce cytokines which recruit and activate macrophages thus promoting further continuation of the inflammatory process (Fig 3.9) (12).

Thus macrophages play a central role in chronic inflammation by – Ingestion of microbes and devitalized tissue, secreting mediators of inflammation, displaying antigens to T-lymphocytes and responding to signals from T lymphocytes resulting in activation of cell mediated immunity (11).

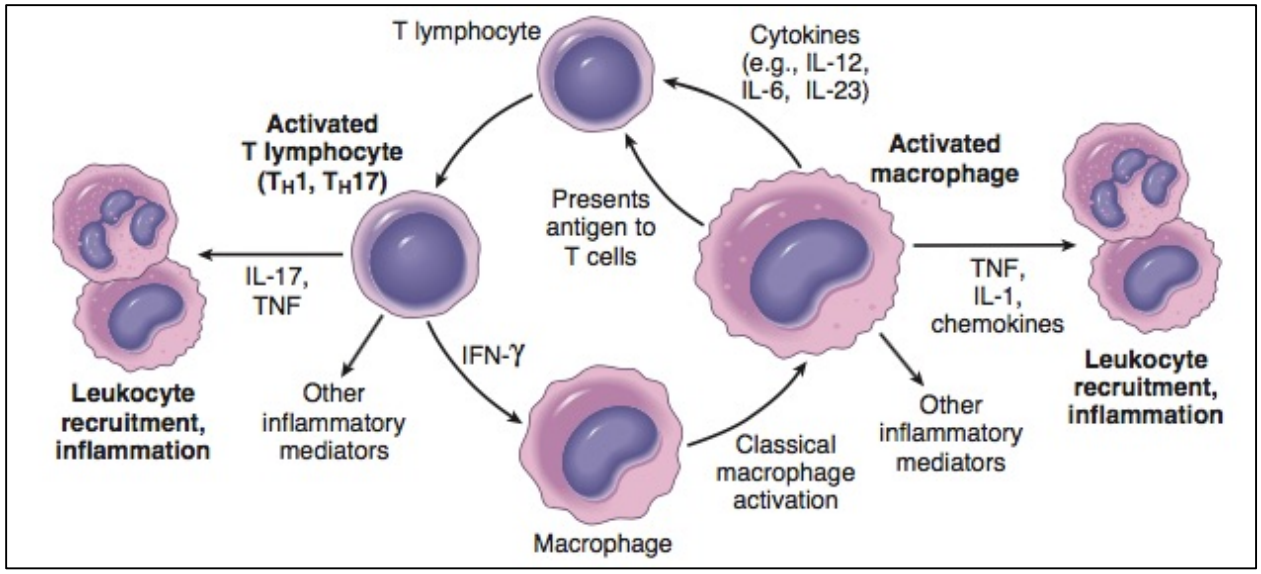

Fig 3.10 Macrophage-Lymphocyte interaction

3.4.5 EPITHELIOID HISTIOCYTES

Mononuclear cells with finely granular eosinophilic cytoplasm, vesicular nuclei, and indistinct cell boundaries usually noted in granulomas are known as Epithelioid histiocytes. Originating from the bone marrow, these cells mature into monocytes, which enlarge and enter peripheral circulation. When recruited into tissues, they are called as histiocytes. The activation of histiocytes, via the innate immune response, gives the cells their characteristic epithelioid

appearance (13). These epithelioid histiocytes are considered as specialized monocytes that are characteristic of granulomas(11).

3.4.6 GIANT CELLS

Multinucleated giant cells are commonly found in granulomas. They are formed by fusion of macrophages which may be caused by simultaneous phagocytosis by two or more macrophages.

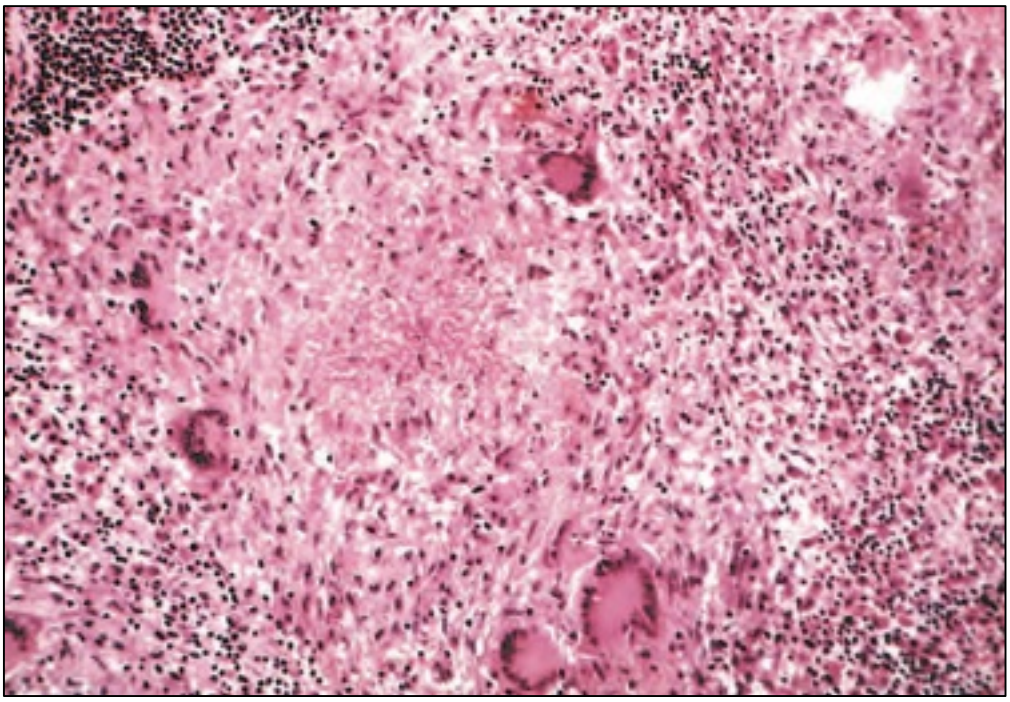

Fig 3.11 Tubercular granuloma with areas of central necrosis surrounded by multiple Langerhan type giant cells, epithelioid cells and lymphocytes

3.4.7 CLASSIFICATION OF GRANULOMATOUS INFLAMMATION

As mentioned previously, Granulomatous inflammation ranges in a wide spectrum ranging from focal well-defined granulomas to diffuse inflammation. Focal granulomas are classified into two major forms – Foreign body granuloma and Immune granuloma.

Foreign-body granulomas are granulomatous reactions to inert material like suture, talc, and food material without a T-cell immune response. A collection of histiocytes are found apposed to the surface of the foreign material as single histiocytes are unable to engulf the foreign material. The inert material can be visualized by light microscopy and often shows birefringence using polarized light.

Immune granulomas induce a persistent T cell–mediated immune response in response to varied etiologies. They may further be classified into Necrotizing and Non-Necrotizing, suppurative and Histiocytic granulomas which is illustrated in the table below(13).

Pattern of inflammation	Associated Etiology
Foreign Body	Talc, starch, suture, hyaluronic acid (and other injectable fillers)
Necrotizing Granulomas	**Infectious**: Coccidioides immitis/C. posadasii, Cryptococcus neoformans/C. gattii, Histoplasma capsulatum, Blastomyces dermatitidis, Aspergillus spp., Mucorales, Mycobacterium tuberculosis , Non-tuberculous mycobacteria, Brucella spp., Nocardia spp., Yersinia spp., Bartonella henselae, Pneumocystis jiroveci, Echinococcus granulosus, xanthogranulomatous pyelonephritis **Autoimmune** : Rheumatoid nodule, granuloma annulare, necrobiosis lipoidica, granulomatosis with polyangiitis Non-Necrotizing Granulomas
Non-Necrotizing Granulomas	**Infectious**: Candida albicans (hepatosplenic candidiasis), C. immitis/C. posadasii, Coxiella burnetii, cytomegalovirus, M. tuberculosis, non-tuberculous mycobacteria including M. leprae (tuberculoid forms), Schistosoma spp., Toxoplasma gondii, Rickettsia spp., Salmonella typhi, hepatitis A & C virii, **Autoimmune** : Sarcoidosis, Churg Strauss, giant cell arteritis, systemic lupus erythematous, Crohn disease, primary biliary cirrhosis, orofacial granulomatosis, rosacea, granuloma annulare **Toxic**: actinic granuloma, berylliosis, zirconium, hot tub lung **Drug**: Bacillus Calmette-Guérin, Non-steroidal anti-inflammatory drugs, antibiotics, methotrexate **Other**: Lymphoid interstitial pneumonia, hypersensitivity pneumonitis, chronic lymphocytic leukemia
Suppurative Granulomas	**Infectious** : Actinomyces spp., Dirofilaria spp., Acanthamoeba spp., Balamuthia mandrillaris, B. henselae, B. dermatitidis, Brucella spp., Chlamydia trachomatis (serotypes L1, L2, L3 causing lymphogranuloma venereum), dematiaceous fungi causing chromoblastomycoses and phaeohyphomycosis, non-tuberculous mycobacteria, Francisella tularensis, Prototheca spp., Sporothrix schenckii, Paracoccidioides brasiliensis, Yersinia spp., Enterobius vermicularis
Histiocytic response,no granulomas	**Infectious** : Tropheryma whipplei, Listeria monocytogenes, non-tuberculous mycobacteria including M. leprae (lepromatous forms), H. capsulatum, Leishmania spp., Rhodococcus spp. (with malakoplakia) **Other** : Langerhans cell histiocytosis, granulomatous mycosis fungoides, juvenile xanthogranuloma, reticulohistiocytoma, Rosai Dorfman, pineal germinoma, seminoma/dysgerminoma, dendritic cell sarcoma, Erdheim-Chester disease, hemophagocytic lymphohistiocytosis, histiocytic sarcoma, interdigitating cell sarcoma, Langerhans cell sarcoma

Table 3.2 Classification of Granulomatous inflammation

3.5 ETIOLOGY OF GRANULOMATOUS INFLAMMATION

Granulomatous inflammation may be caused by a wide array of etiologies which include infectious, allergic, toxin-related, autoimmune, neoplastic or unknown etiology (13).

3.6. GRANULOMATOUS MASTITIS

3.6.1 HISTORICAL PERSPECTIVE AND DEFINITION

In a case series published in 1972, Kessler and Wolloch from Israel described 5 cases of young women presenting within 5 years of childbirth, with a breast lump that was clinically suggestive of malignancy. Histopathological analysis of these lesions showed presence of non-specific granulomas comprising giant cells and epithelioid histiocytes, confined to the lobules. They noted the distinct nature from other inflammatory mastitis, lack of response to antibiotics, response to steroid therapy and raised the possibility of auto-immune etiology. This was the first description of a newly described entity called Granulomatous mastitis (2).

3.6.2 DISEASE BURDEN AND GEOGRAPHIC DISTRIBUTION

The exact disease burden of granulomatous mastitis is difficult to ascertain as the number of reported cases vary and most of the published literature comprises of isolated case studies and

case reports. The annual prevalence of IGM was reported to be 2.4 per 100,000 women aged 20-40 years, in a review article on Idiopathic Granulomatous mastitis among Hispanic women(14).

Our literature search of PUBMED database using the keywords “Idiopathic granulomatous mastitis”, “Granulomatous lobular mastitis” and “Granulomatous mastitis revealed 348 published articles since its initial description in 1972.

In a review article published in 2014 by Altintoprak et al, the distribution of large case series described in literature (1995-2014) was mostly from the Mediterranean and South Asian region (15). The most number of cases were reported from Turkey (>200 cases), followed by China and South Korea. Number of cases were reported from USA were 126 whereas France had the highest number of cases from Europe (55 cases). Total of 36 cases were reported from India. However, this distribution was not based on ethnicity and was rather based on purely geographic distribution.

3.6.3 EPIDEMIOLOGY

Though most commonly found in women, some cases of granulomatous mastitis in males have also been described. Granulomatous mastitis is most commonly found in young women of the childbearing age group, usually occurring within 5 years of childbirth(16–20).

In a large multicentre retrospective study involving 720 patients published in 2017, mean age of patients was found to be 36 years (age range 32-42 years) (21). Omranipour et al, in a retrospective review of 43 cases in Iran, found patients with Granulomatous mastitis were within

an age range of 24-55 years with the mean age being 33.5 years (22). In a retrospective review of 18 patients over a 25 year period, median age of patients was 36 years and 56% of these women were within 5 years of childbirth (23).

3.6.4 ETIOPATHOGENESIS

Granulomatous mastitis despite having been described four decades ago is not well understood. Its etiology and factors involved in its pathogenesis have not been completely studied or described in literature.

Etiological factors that have been proposed include factors such as hormonal imbalance, autoimmunity, infections, α1-antitrypsin deficiency, smoking, ethnicity etc. (15). The three main hypothesis that have been proposed to explain its etiopathogenesis is Autoimmunity, Infections and Hormonal imbalance (24).

3.6.4.1 AUTOIMMUNITY AND AUTOINFLAMMATION

The most widely accepted etiological theory is autoimmunity. It has been postulated that IGM is possibly caused by a localized (25) or systemic immune response. This in turn results in a state of autoinflammation.

Autoinflammatory disorders are a distinct group of disorders with recurrent attacks of inflammation without any evidence of exposure to an auto-antigen (24). They may range from

syndromes presenting with recurrent fever to other disease such as Behcets syndrome, Crohn's disease etc. They may be precipitated by a number of factors including environmental factors, psychological factors, trauma, immunization, cold exposure, and dietary indiscretion. Similarly, IGM is characterized by recurrent inflammation of the breast tissue which is characteristic of autoinflammatory disorders.

Omranipour et al postulated a hypothesis that extravasation of milk into the interstitial tissue triggers a localized immune response that results in granulomatous inflammation (Fig 3.11) (22).

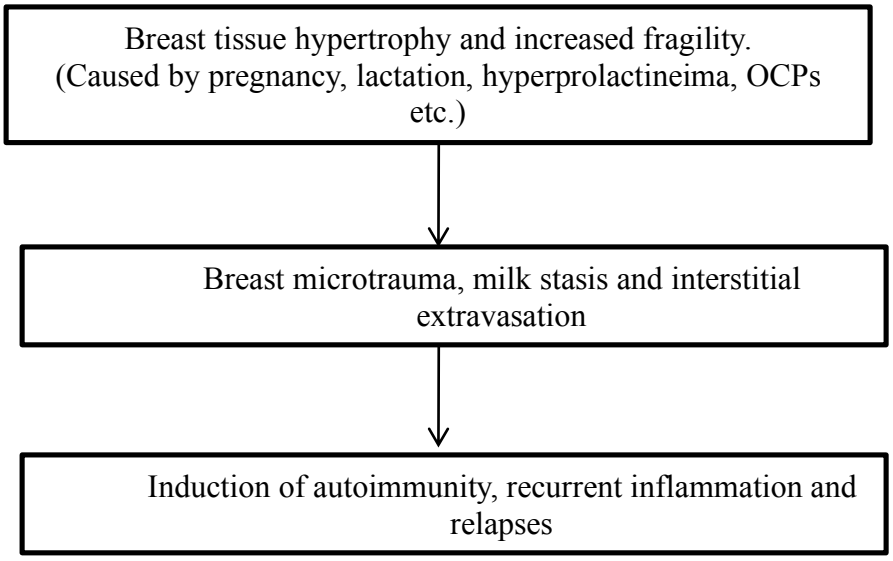

Fig 3.12

There have been various reports of patients with IGM presenting with extra-mammary manifestations such as erythema nodosum, polyarthritis etc. (26–30). In a retrospective analysis of IHC data from biopsy of 18 patients with IGM, Erhan et al, reported T-cell predominance in 14 patients and presence of B-cells in 7 patients (19).

Evaluation of serological markers has also been done in patients with IGM. Ozel et al analyzed Rheumatoid factor (RF), Antinuclear Antibodies (ANA) and Anti-DsDNA in 8 patients with IGM which showed 6 patients positive for RF and 2 patients positive for ANA and Anti-DsDNA (31). In a study by Altintoprak et al, 26 patients with IGM were studied for serum ANA and extractable nuclear antibodies – 9 patients were found to be ANA positive at 1:100 dilution (32). The use of immunosuppressants like corticosteroids, methotrexate, azathioprine etc. in the management of IGM also lends support to the possibility of an autoimmune etiopathology (33–35).

3.6.4.2 MICROBIOLOGICAL AGENTS

Several authors have investigated the hypothesis that Granulomatous mastitis may be caused by infectious agents that cause chronic, indolent and persistent infection in the breast tissue. The spectrum of causative organism includes Bacterial, Mycobacterial, Fungal and Parasitic organisms.

BACTERIAL ORGANISMS

Thornton et al, in a study assessing the endogenous flora of the human breast found that 53% of specimens obtained from 231 cultures were positive for coagulase-negative staphylococcus. Aerobic bacteria such as Diphtheroids, Lactobacillus, D-Enterococcus, Micrococcus, Alpha-

haemolytic Streptococcus and anaerobic organisms such as Propionibacterium acne, Peptococcus and Clostridium sporogenes were most commonly cultured (36).

Taylor et al showed the presence of Corynebacterium in 34 patients with IGM(37). Subsequent studies by Paviour and other authors showed presence of Corynebacterium species(38). In a metagenomic review published by Hai-jing Yu et al, 13 out of 19 patients with IGM grew Corynebacterium, the predominant organism being Corynebacterium kroppenstedtii (39). However, there is difficulty in differentiating if the presence of these organisms in culture signifies, contamination, colonisation or true infection. Large case series have not shown significant growth of bacterial organisms. Retrospective analysis of 66 patients presenting to Department of Endocrine Surgery CMCH, Vellore from 2011-2015 showed growth of Coagulase-negative Staphylococcus aureus in 8 patients and Enterococcus, Citrobacter diversus and Staphylococcus aureus in 1 patient each (Unpublished data).

MYCOBACTERIAL ORGANISMS

Propensity for mycobacterial organisms to cause chronic granulomatous inflammation has led to the proposition that Mycobacterial organisms may be involved in the etiopathogenesis. Incidence of tuberculous mastitis accounts for less than 1% and less than 0.1% of benign breast lesions. Mycobacterium tuberculosis is the most common organism, however, atypical mycobacteria have been found in isolated cases (40). In retrospective data from our institution from 2011-2015, 1 out of 66 patients grew Mycobacterium chelonae (unpublished data).

FUNGAL ORGANISMS AND PARASITES

There have been very few published case reports on growth of fungal and parasitic organisms in Granulomatous mastitis. Blastomycosis, Cryptococcosis, Histoplasmosis, Actinomycosis and filarial organisms such as Wuchereria bancrofti have been described to cause granulomatous inflammation in breast tissue(15,41–45). In our retrospective analysis, there were no patients who grew fungal organisms or showed presence of parasitic organisms.

3.6.4.3 HORMONAL IMBALANCE

Prevalence of the disease most commonly among young women, especially within 5 years of childbirth and recent history of lactation has given rise to the theory of hormonal imbalance being a possible etiological factor in Granulomatous mastitis.

The breast is increasingly being considered as an endocrine organ, in its current context. This organ, not only undergoes dynamic change during different phases of the menstrual cycle but also undergoes numerous structural and functional changes during pregnancy and lactation. As described above, estrogen and progesterone are important for development of the ductal and lobular system respectively. While estrogen has a tendency to promote mitoses and proliferation, progesterone has a biphasic effect- initially promoting mitosis and subsequently slowly down the same by causing cell cycle arrest in early G1 phase. Proliferation of breast cells, increase in breast volume, size, increase in vascularity, increase in secretion etc. are few of the changes that may cause increased propensity to breast infections and mastitis.

OCPs cause prolongation of cellular proliferation, thereby leading to a hypothesis of possible etiological role in IGM. Definitive causative association with OCP intake has been questioned by several authors. In a study by Al-Khaffaf et al, 18 patients with Granulomatous mastitis were analyzed and history of OCP intake was found in 27.7% (23). Asoglu et al studied 18 patients with Granulomatous mastitis and 22.2% showed history of OCP intake (17). However, Baslaim et al in a review of 20 patients showed no use of OCPs (18).

As suggested by Omranipour et al, hormonal alterations due to various factors such as pregnancy, lactation, use of oral contraceptive pills (OCP) etc. has been thought to increase breast secretions and alter the breast tissue environment, thereby making it prone for extravasation of intraluminal contents causing a local autoimmune phenomenon resulting in granulomatous inflammation.

However, occurrence of disease in non-lactation women and in male breasts has led to the conclusion that hormonal factors may not be solely responsible.

3.6.4.4 SMOKING

Some authors believe that granulomatous mastitis has similarities to periductal mastitis and may even be considered as components of the same pathology. Pathogenesis of periductal mastitis has been attributed to stagnation of secretions within the ductal system due to either squamous metaplasia or duct dilatation which may occur as part of aberrations of normal development. This causes periductal inflammation and duct fibrosis causing extravasation of intraluminal contents into the interstitial tissue. This subsequently leads to bacterial colonization.

Smoking as an etiological risk factor has been extensively studied in periductal mastitis. Shafer et al showed that the relative risk of developing recurrent subareolar breast abscess was 9.2 (3.6-23.5) for a light smoker and 26.4 (9.9-70.2) for a heavy smoker (46).

Heavy smokers were more likely to harbour anaerobic bacteria and also have a higher degree of squamous metaplasia in the ducts. Toxic products have also been seen in secretions in smokers. These may cause extravasation of intraluminal secretions as there is a greater propensity for duct epithelial damage facilitating extravasation. Anti-estrogenic effect of smoking also has been postulated to cause earlier menopause and inhibit growth of Gram-positive bacteria thus facilitating growth of anaerobic bacteria.

However there has been no definite association between smoking and granulomatous mastitis. Al-Khaffaf et al showed history of current smoking in 17% of patients with IGM as compared to 60% in periductal mastitis group. 22% of patients with IGM had past history of smoking as compared to 69% in periductal mastitis group (23). Asoglu et al showed 77% (14/18) patients with history of nicotine addiction (17). Baslaim et al did not have any patients in their series with history of smoking (18). In a large case series of 720 patients published by Uysal et al, 24% of patients had history of smoking (21).

3.6.4.5 HYPERPROLACTINEMIA

Elevated prolactin levels have been found to play a role in inflammation, fibrocystic changes, ductal ectasia, benign breast lesions, IGM and even in the development of breast carcinoma. There have been case reports of patients with hyperprolactinemia, either due to pituitary

adenoma or drug-induced, presenting with IGM(47–51). Hence hyperprolactinemia has been postulated to play a probable role in its etiopathogenesis.

3.6.4.6 ETHNICITY

Altintoprak et al looked at the worldwide distribution of IGM cases published in literature. This showed a predominance of cases in the Mediterranean and South East Asian regions (15). This peculiar distribution of cases has given rise to an epidemiological conundrum of the possibility of ethnicity or any common environmental exposure that may be responsible in disease causation.

3.6.4.7 MISCELLANEOUS

In a case report by Schelfout et al, the authors demonstrated Alpha-1 anti-trypsin deficiency (52). Alpha-1 anti-trypsin is a protein that is synthesized in the liver. It is a serine-protease inhibitor that prevents activation of protease and helps in control of inflammation. Deficiency of this protein has been attributed to the cause of inflammation in a number of inflammatory diseases like bronchiectasis etc.

3.6.5 CLINICAL PRESENTATION

Granulomatous mastitis has a wide spectrum of clinical presentation. The disease occurs most commonly in women, though few cases of male patients have been described in literature (53). Though there is a wide age range of patients, most common age group affected is second to fourth decade (15,18–23). The most common presentation is an ill-defined firm mass in the upper outer quadrant of the breast in a young woman with recent childbirth or lactation. As described in the index case report, it commonly mimics malignancy and is often diagnosed after a core biopsy for suspected malignancy. Patients may often present with signs of inflammation such as warmth, erythema, tenderness, ulceration of overlying skin with pus discharge and multiple discharging sinuses (Fig 3.6). In the case series published by Kiyak et al, 66.6% of patients presented with inflammatory signs. Axillary lymphadenopathy was seen in 42% (54).

Fig 3.13 Clinical photograph of IGM with signs of inflammation and multiple sinus tracts

3.6.6 DIAGNOSIS – IMAGING AND PATHOLOGY

Radiological evaluation of women with granulomatous mastitis maybe done by Ultrasound or Mammogram. Larsen et al retrospectively analysed the imaging characteristics in 54 women diagnosed with granulomatous mastitis. The most common ultrasound feature was irregular hypoechoic mass associated with multiple tubular hypoechoic structures (59%). Features of skin edema, thickening and sinus tracts are also encountered in 52%. Axillary lymphadenopathy was seen in 28%.

The most common mammogram feature was large focal asymmetric density (44%) with axillary lymphadenopathy in 18% and skin thickening in 7%(55). Though radiological features may often be equivocal and non-contributory, mammogram and ultrasound are often the first step in work up of patients with granulomatous mastitis. Features suspicious of malignancy on imaging warrant further evaluation with histopathology.

Diagnosis of granulomatous mastitis is confirmed by histopathology with tissue obtained either by excision or core biopsy. Inflammation in granulomatous mastitis is predominantly localised to the regions of the lobules (14). Histopathology shows granulomas with Langerhans giant cells, epithelioid histiocytes surrounded by lymphocytes. Presence of non-caseating granulomas composed of epithelioid histiocytes and multinucleate giant cells is characteristic of idiopathic granulomatous mastitis as compared to Tubercular mastitis where there is caseous necrosis. Inflammatory cells such as neutrophils, plasma cells and lymphocytes is present. Special staining for acid fast bacilli (Ziel Neelsen staining) and fungal organisms (PAS, Giemsa) are often negative ruling out infective causes. Tissue culture for Bacterial, Mycobacterial, Fungal

organisms may also be used as an adjunct to rule out infectious etiology. Presence of acini and other lactational changes may be found in certain regions. Occasionally, there may be dilated ducts with periductal or intraductal inflammation. In a clinicopathological study of 26 patients, by Tse et al observed granulomas in all patients. 17 patients had Langerhans giant cells and four showed foreign body giant cells. Majority of the inflammatory cells were lymphocytes (>65%) (16).

Confluent granulomatous lesions may result in the formation of micro abscesses. Occasionally, there are microcystic space that are noted in the centre with no secretions. Neutrophils may be found within the space or outlining it.

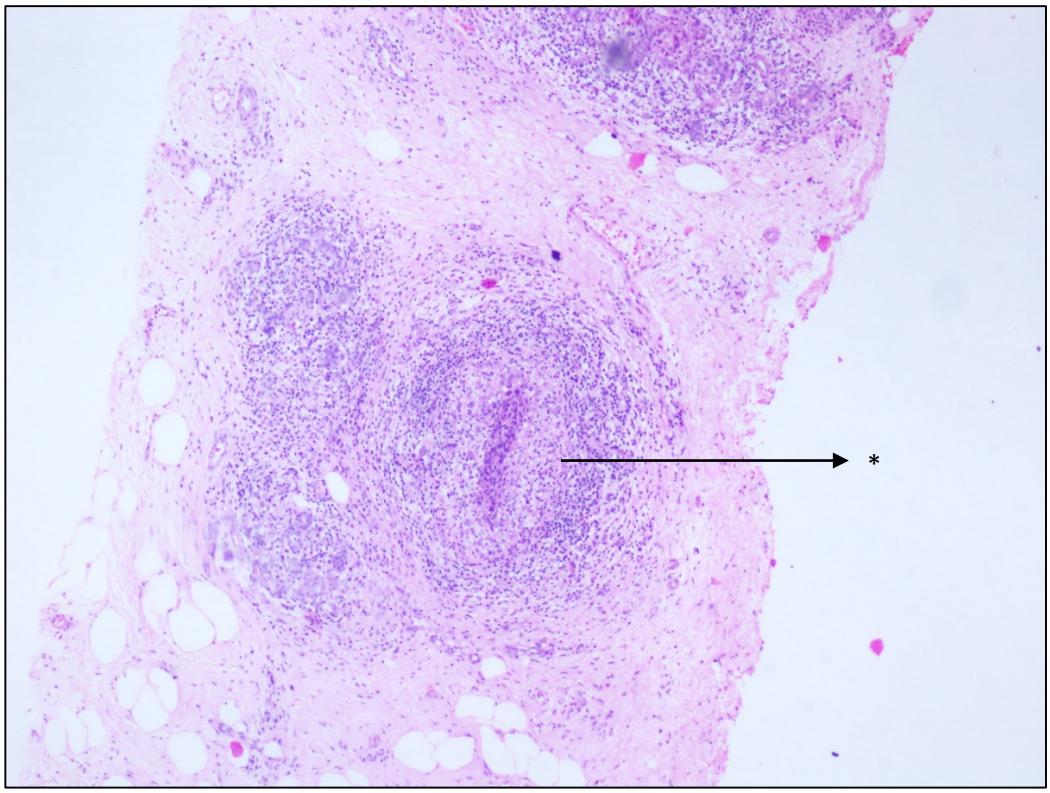

Fig 3.14 *Lobulocentric granulomatous inflammation – Low magnification

(Courtesy: Department of Pathology, CMC, Vellore)

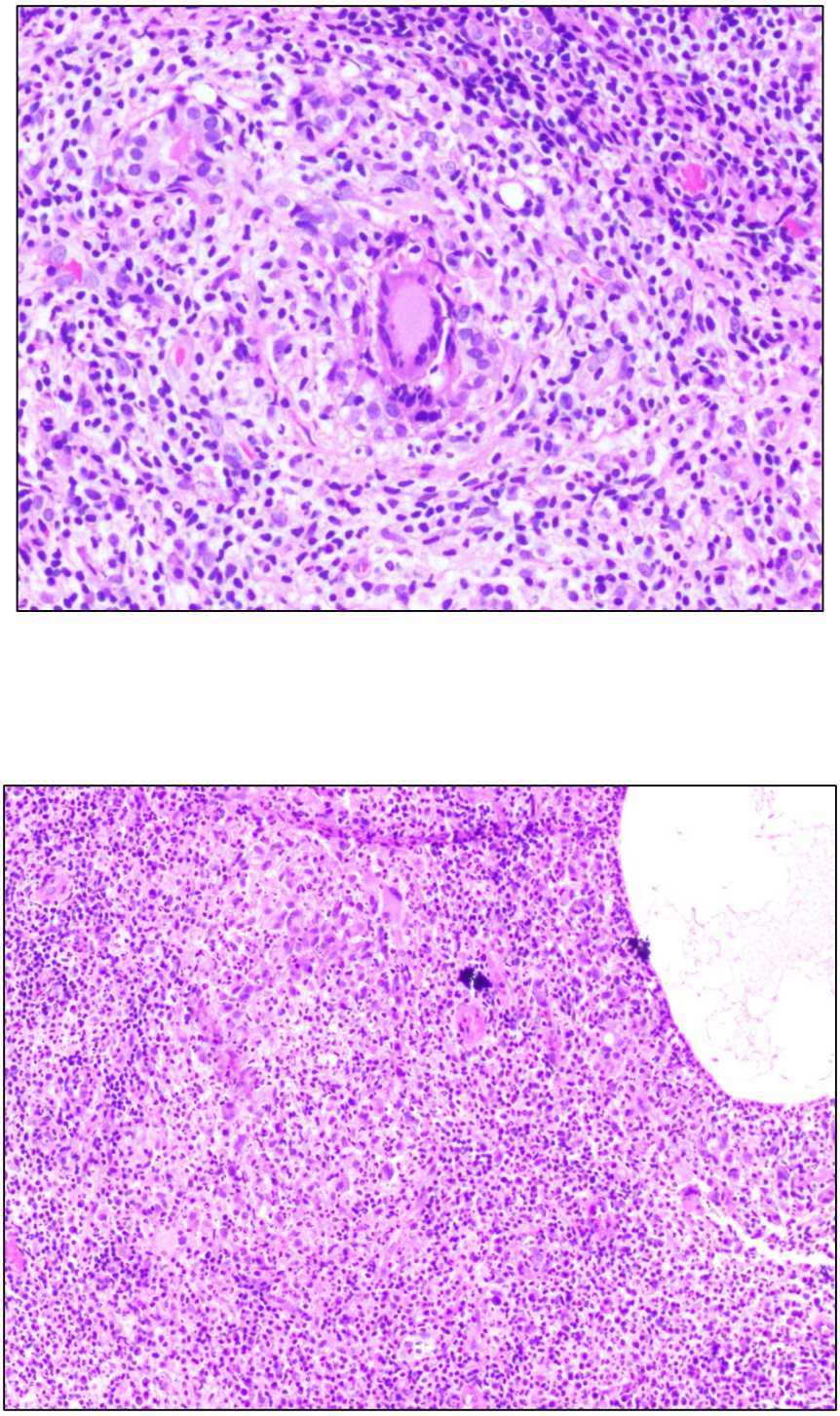

Fig 3.15 Perilobular inflammation with multinucleate giant cells, epithelioid histiocytes and lymphocytes among acini (Courtesy: Department of Pathology, CMC, Vellore)

3.6.7 MANAGEMENT

Both medical and surgical therapy or a combination of both are recommended for management of granulomatous mastitis. Localized lesions with no signs of skin changes or sinuses are amenable to wide local excision of these lesions. Extensive abscess formation, presence of complex sinuses and skin involvement is often managed medically initially or with a combination of both. Surgical excision in these conditions are more prone for recurrence and do not provide cosmetically acceptable results after debridement or excision.

Corticosteroids are the principal form of medical therapy. Described in 1980 by Dehertogh et al, steroid therapy has caused a paradigm shift in management of granulomatous lesions from completely surgical to primarily medical (34). Though initial authors prescribed larger doses of steroids for shorter durations, it is now recommended to use smaller doses for steroids for longer periods lasting 3-6 months to reduce recurrence of disease. Other immunosuppressive agents such as Methotrexate, Azathioprine etc. may also be used in steroid resistant cases. Fig 3.14 represents a treatment algorithm for granulomatous mastitis (56).

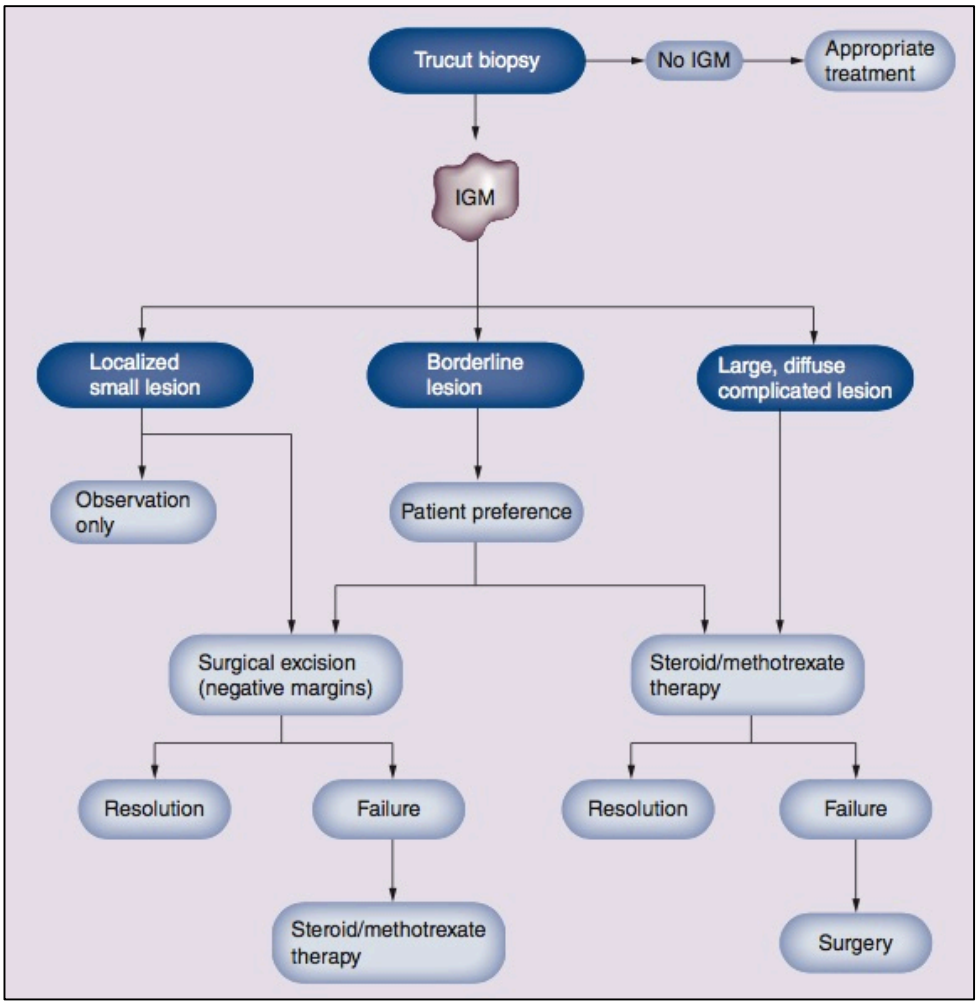

Fig 3.16 Diagnostic and treatment algorithm

4. MATERIALS AND METHODOLOGY

The study was conducted after obtaining approval from the Institutional Review Board and Ethics Committee of Christian Medical College, Vellore.

Study Design

The study was designed as a prospective case control model.

Inclusion Criteria

1. **Cases:** All patients diagnosed with Granulomatous Mastitis based on histopathology from either core biopsy or excision biopsy.
2. **Controls:** Normal women without Granulomatous Mastitis who have been group matched with individual cases for age (Age +/- 5 years) and childbirth (Last childbirth <5 years).

Exclusion Criteria

1. Patients with histopathology other than Granulomatous Mastitis.
2. Patients not consenting to be part of the study.

Study population

Cases and controls were recruited from the Outpatient department and wards of Department of General Surgery, Endocrine Surgery and Obstetrics & Gynecology.

Informed Consent Administration

Both cases and controls were given an information sheet with details about the study in English and in translated versions in Tamil, Telugu, Hindi and Bengali. Informed consent was taken by the principal investigator from the patient after being given adequate opportunity to read the information sheet and ask questions.

Methodology of Data collection:

Data collection was done solely by the principal investigator. All patients with a clinical suspicion of Granulomatous mastitis underwent core biopsy. Samples were obtained for both histopathology as well as cultures. All cases and controls satisfying inclusion criteria, after obtaining informed consent underwent a one-on-one interview by the principal investigator with the help of a structured, validated questionnaire detailing the patient demographics, clinical profile and presence of risk factors. Subsequently, serum sample was obtained from them and was analyzed for the following

1. Serum Anti-Nuclear antibody (ANA)
2. Serum Prolactin
3. Serum Globulin

If the patient was ANA positive, the sample was further processed for specific autoantibody analysis. Similarly, if the globulin levels were elevated, the sample was further processed for analysis of Immunoglobulin levels (IgA, IgM, IgG).

Methodology of Laboratory analysis:

1. Serum ANA estimation was done by indirect immunofluorescence using fluorescein coated antibodies to human IgG (EUROIMMUNE, Germany) for both cases and controls.
2. Serum specific autoantibody estimation was done by Enzyme linked Immunosorbent Assay only in the cases, if Serum ANA was positive.
3. Serum globulin levels was derived from A/G ratio (Albumin measured by Bromocresol green dye binding colorimetric assay and total Protein measured by biuret method) for both cases and controls.
4. Immunoglobulin levels was estimated using immunoturbidometry in both cases and controls, if serum globulin levels were elevated.
5. Serum prolactin was estimated using sandwich immunoassay in both the cases and controls.
6. Tissue taken at the time of core biopsy or excisional biopsy was sent for Bacterial, Fungal, MGIT culture and Gene XPERT TB PCR

Interpretation of results:

1. Serum ANA was considered positive if indirect immunofluorescence showed fluorescence intensity more than 2+ at a dilution of 1:100. If serum ANA was positive, specific autoantibody analysis (Eg. AntiSm, AntiRo, AntiLa) was done based on pattern

of immunofluorescence.

2. Serum globulin more than 3.5 gm/dl was considered as elevated and serum IgM, IgA and IgG estimation was done. Serum immunoglobulin levels above the upper limit of normal range was considered as elevated (IgA - >420 ng/ml; IgG - >1700 ng/ml; IgM - >190 ng/ml).
3. Serum prolactin was considered as elevated if levels were more than 25 mg/dl.

5. STATISTICAL METHODS

Sample size calculation

Assuming power of the study is 80%, confidence interval of 95%, proportion of ANA positives in comparison group is 5%, using the formula,

$n= (r+1/r)\ (p^*)\ (1-p^*)\ (Z\beta + Z\alpha/2)^2 \div (p1-p2)^2$

p_1 - Proportion of cases with ANA positivity

p_2 - Proportion of controls with ANA positivity

$Z\alpha/2$ - Standard normal variate for level of significance

$Z\beta$ - Standard normal variate for power

r - Proportion of cases to controls

$p^* = p_1 + p_2 \div 2$

Two-sided confidence level(1-alpha)	95
Power (% chance of detecting)	80
Ratio of Controls to Cases	1
Hypothetical proportion of controls with exposure (57)(58)	5

Hypothetical proportion of cases with exposure(32) 35

Least extreme Odds Ratio to be detected: 10.23

Category	Kelsey(59)	Fleiss(60)	Fleiss with CC
Sample size cases*	28	27	34
Sample size controls*	28	27	34
Total sample size*	56	54	68

CC – Continuity correction

* Results from OpenEpi, Version 3, open source calculator--SSCC

Data Entry and Analysis

Data collected from all cases and controls were entered using EpiData Manager and EpiData Entry Client (v4.2.0.0). Analysis was done using STATA Data analysis and statistical software (v13.1).

All demographic and clinical variables were summarised as counts and percentages for categorical variables, mean and standard deviation for symmetrically distributed continuous variables and median and range for skewed continuous variables.

Chi square test was used to compare the proportions between categorical variables. The Independent t-test was used to compare the means between two groups for normally distributed continuous variables and the Mann-Whitney U test was used for skewed variables. For all data analysis, 5% level of significance was considered to be significant.

6. STUDY ALGORITHM

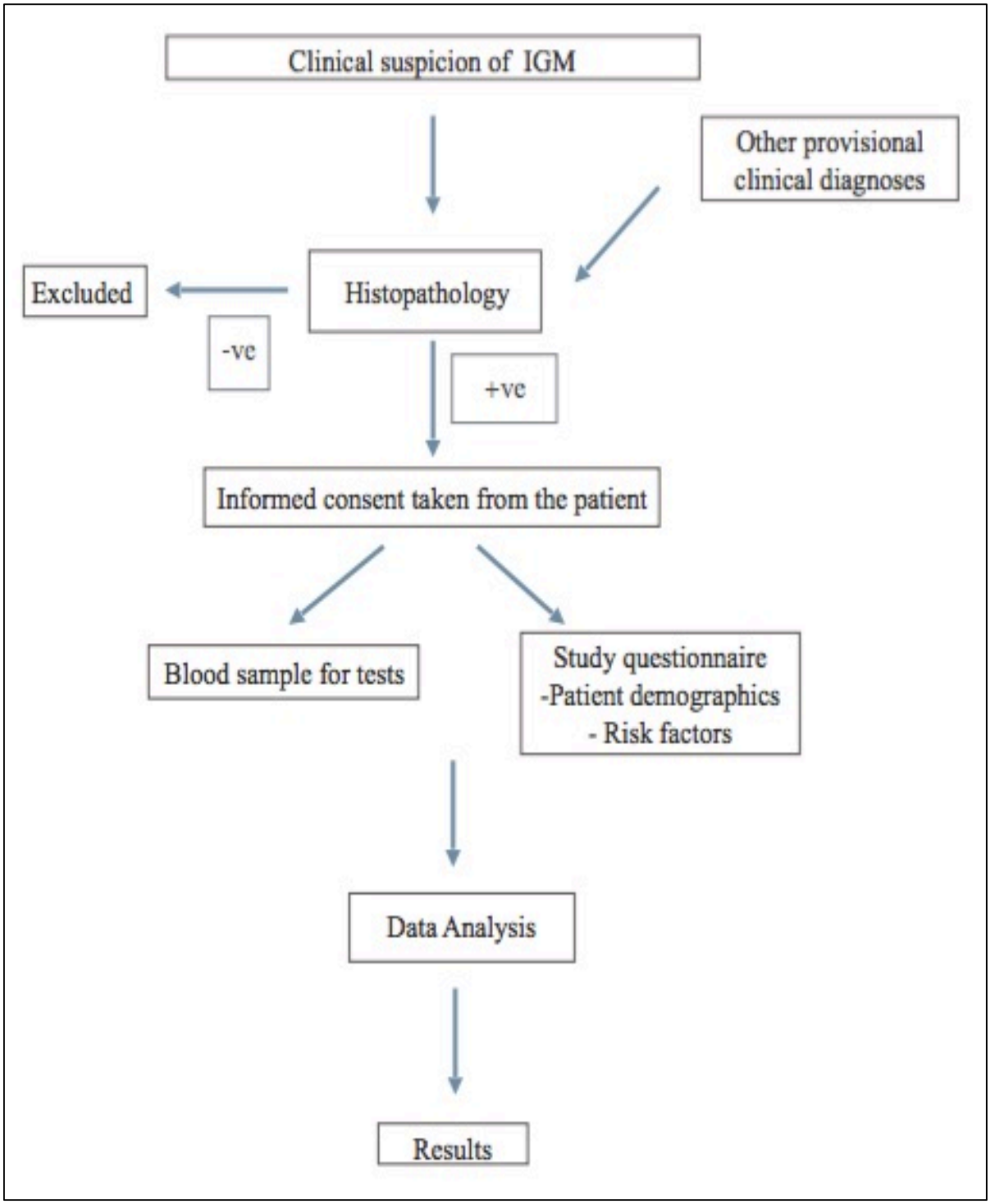

Fig 6.1

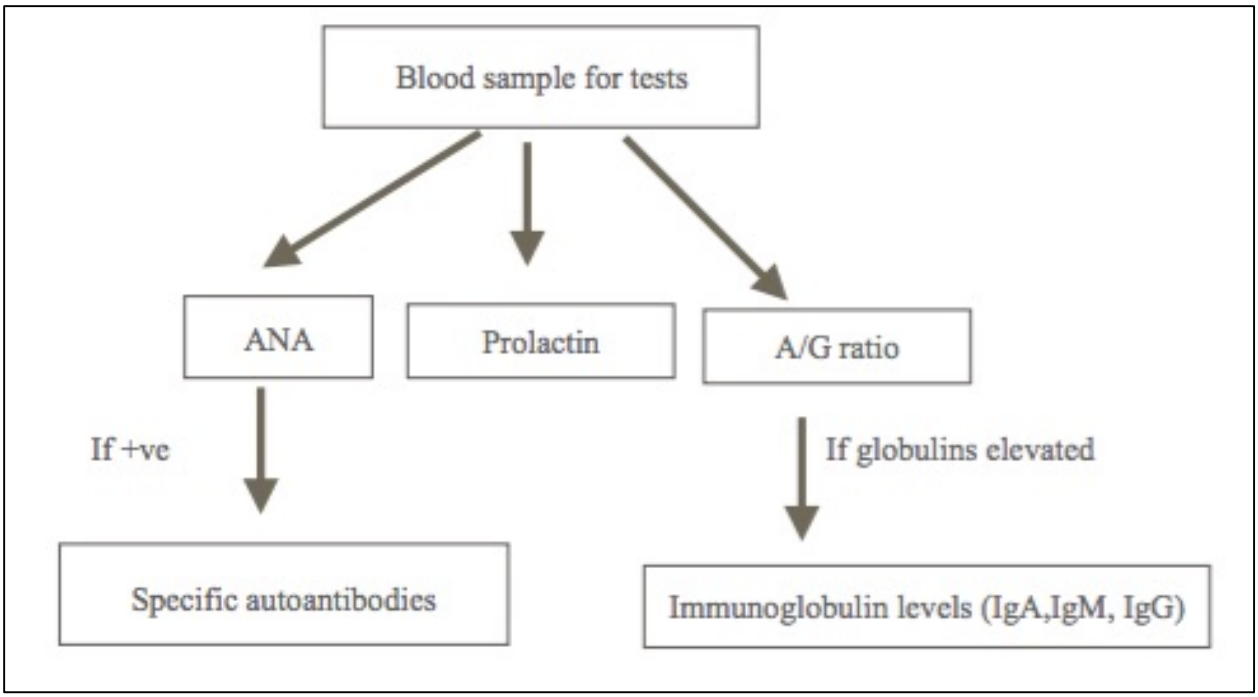
Blood sample for tests
ANA
Prolactin
A/G ratio
If +ve
If globulins elevated
Specific autoantibodies
Immunoglobulin levels (IgA,IgM, IgG)

Fig 6.2

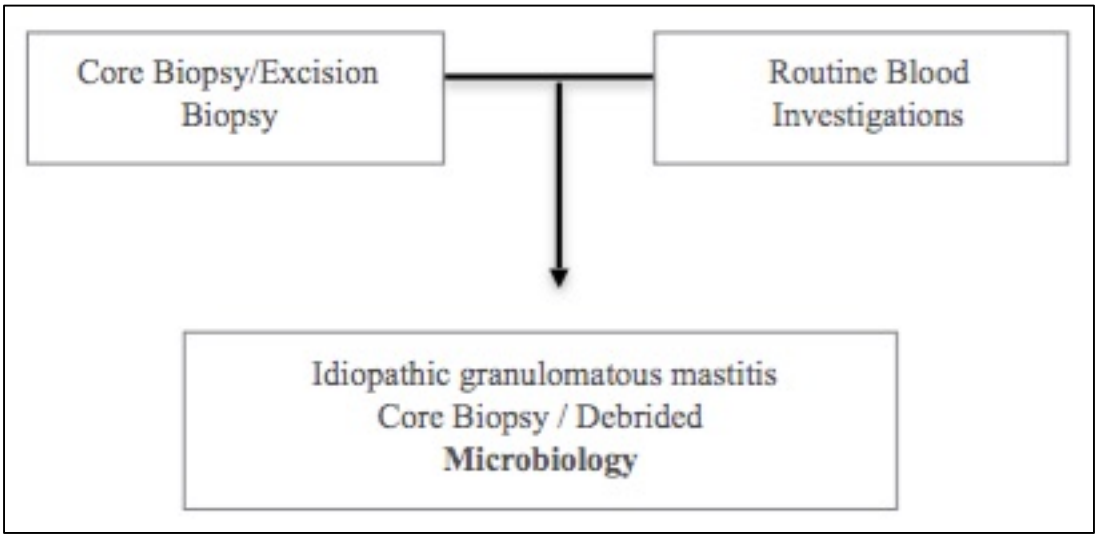
Core Biopsy/Excision Biopsy
Routine Blood Investigations
Idiopathic granulomatous mastitis
Core Biopsy / Debrided
Microbiology

Fig 6.3

7. RESULTS

The total number of cases of histologically proven Granulomatous Mastitis recruited in the study period from May 2016 to August 2017 (1 year and 3 months) was 30 cases. As detailed in the study algorithm, 30 normal women who were matched for the same age group (+/- 5 years) and last child birth (+/- 5 years from last child birth) were recruited as controls.

Category	Number of patients (n)
Cases	30
Controls	30

Table 7.1

PATIENT DEMOGRAPHICS

All cases and controls in this study were females. Mean age of patients in the case group was 33.76 years (Range 23-62 years) whereas in the control group it was 33.86 years (Range 21-65 years).

Variable	Case (Mean +/- S.D)	Control (Mean +/- S.D)	p value
Age	33.76 +/- 7.68	33.86 +/- 9.23	0.9638

Table 7.2

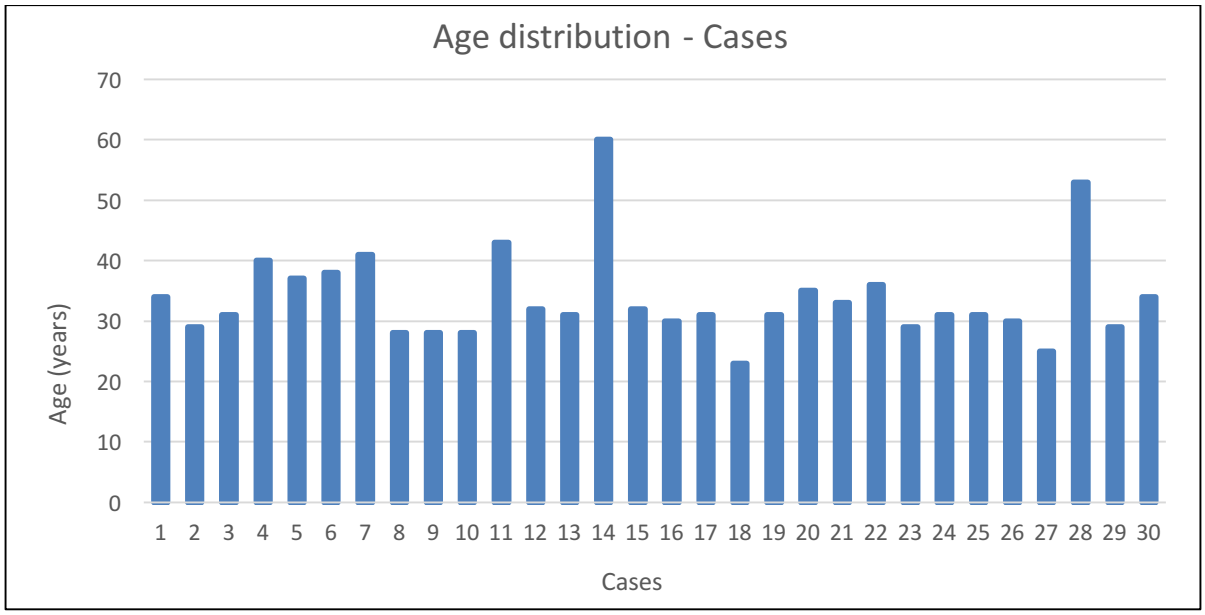
Age distribution - Cases
70
60
50
40
30
20
10
0
Age (years)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Cases

Fig 7.1

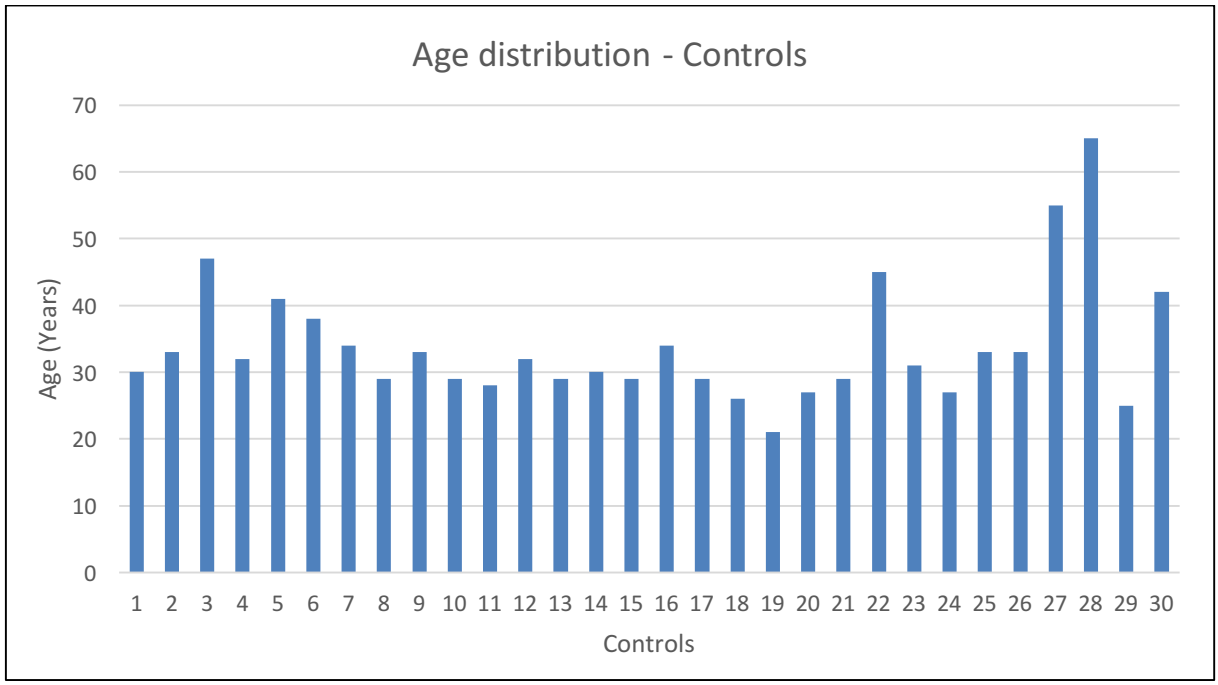
Age distribution - Controls
70
60
50
40
30
20
10
0
Age (Years)
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Controls

Fig 7.2

Majority of the patient population was Indian except for two patients from the case group and three patients from the control group who hailed from Bangladesh.

Distribution of patient population affected by the disease was mostly from West Bengal accounting for 43.3% as shown below.

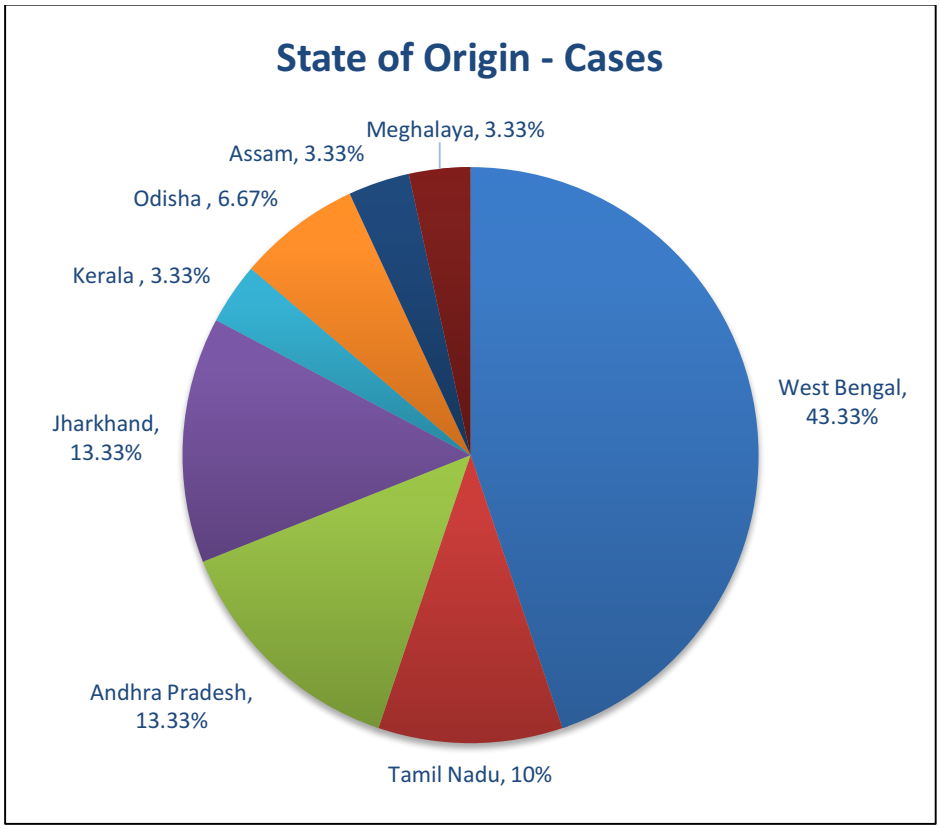

Fig 7.3

State	Cases	Controls	Total
West Bengal	13 (43.3%)	5 (16.67%)	18 (30%)
Tamil Nadu	3 (10%)	18 (60%)	21 (35%)
Andhra Pradesh	4 (13.3%)	2 (6.67%)	6 (10%)
Jharkhand	3 (10%)	0	3 (5%)
Kerala	1 (3.33%)	2 (6.67%)	3 (5%)
Odisha	2 (6.67%)	0	2 (3.33%)
Assam	1 (3.33%)	0	1 (1.67%)
Meghalaya	1 (3.33%)	0	1 (1.67%)
Other country	2 (6.67%)	3 (10%)	5 (8.33%)
Total	30	30	60

Pearson Chi^2 p value –0.004

Table 7.3

INFECTIOUS ETIOLOGY

Six cases showed growth in bacterial culture. However, there was no growth noted in MGIT or Fungal culture and no patients showed positivity on Gene XPERT TB PCR.

Bacteria cultured include Coagulase negative staphylococcus aureus (CONS) (n=5) and Methicillin Resistant Staphylococcus Aureus (n=1). CONS was considered to be a skin contaminant with no pathological significance.

	Bacterial Culture	MGIT Culture	Fungal Culture	Gene XPERT TB PCR
No. of cases positive	6	0	0	0

Table 7.4

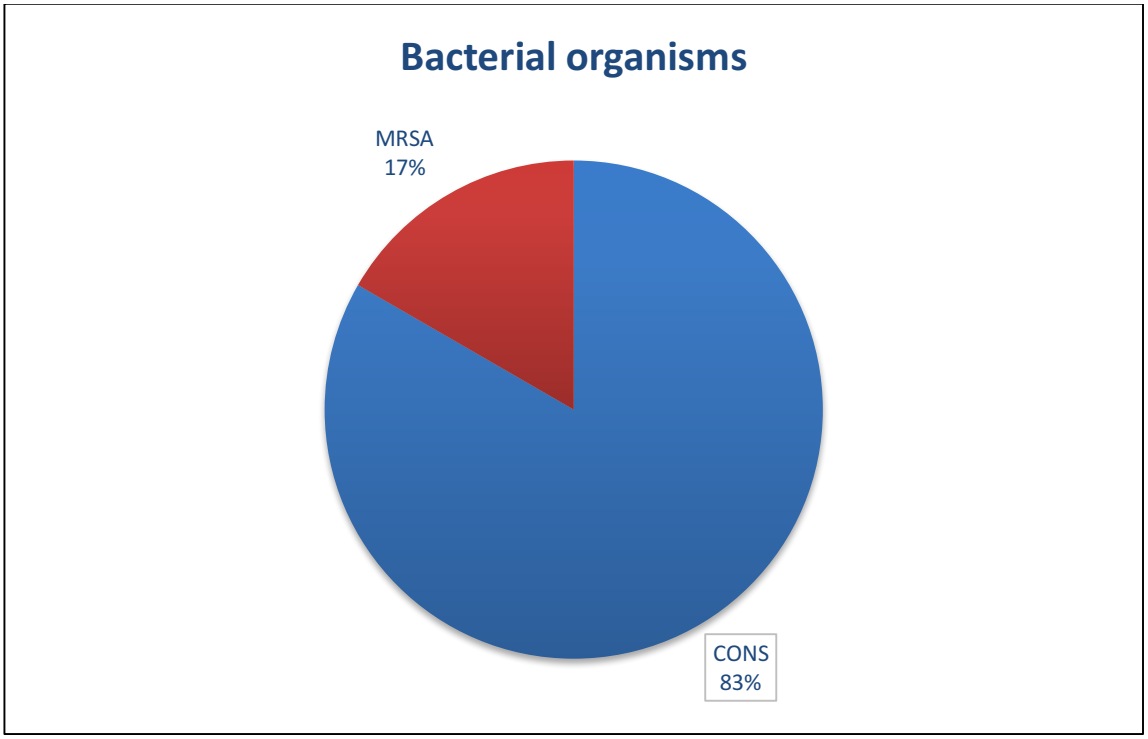

Fig 7.4

AUTOIMMUNE MARKERS

Only one patient in our cases series was Serum ANA positive whereas all controls were ANA negative. This patient was however previously diagnosed with Rheumatoid arthritis and was on treatment for the same with oral steroids, Methotrexate and Leflumide. Specific autoantibody analysis was not done due to inadequacy of the sample.

13 out of 30 cases had an elevated Serum Globulin level as compared to 7 out of 30 controls. Further Serum Immunoglobulin level analysis was done for these patients. IgM levels were not elevated in any of the cases, however, one patient from the control group had an elevated IgM level. None of the cases or controls had elevated IgG or IgA levels.

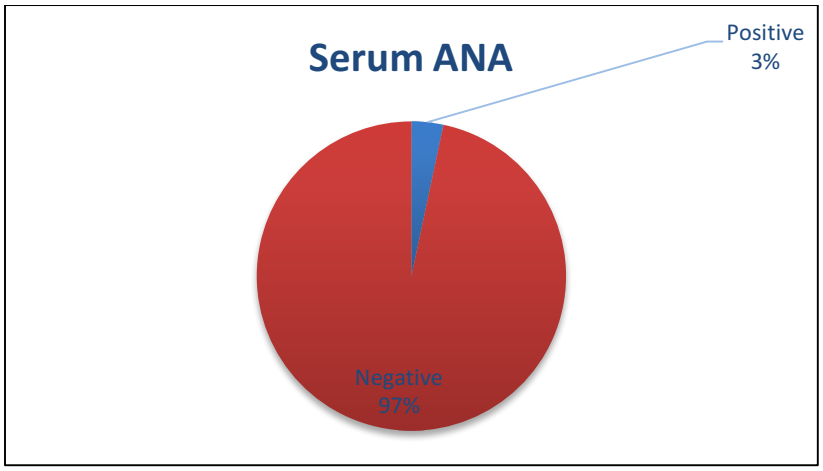

Fig 7.5

Variable	Case (Mean +/- S.D)	Control (Mean +/- S.D)	p value
Serum Globulin level	3.4 +/- 0.67	3.26 +/- 0.36	0.9531

Table 7.5

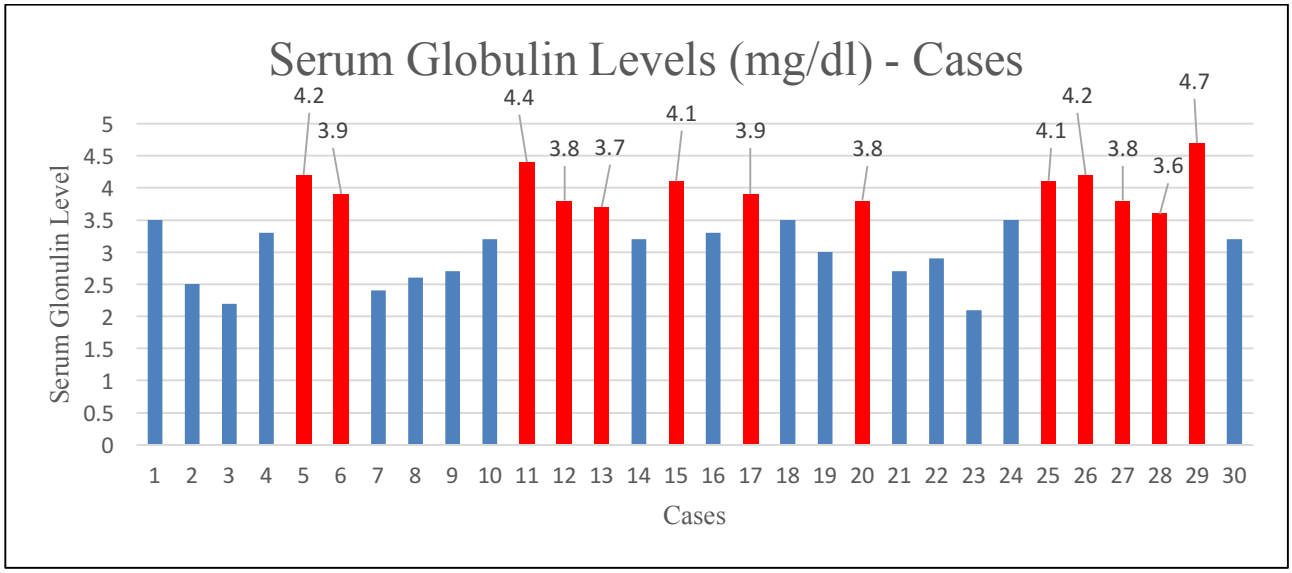
Serum Globulin Levels (mg/dl) - Cases
Serum Glonulin Level
Cases
4.2
3.9
4.4
3.8
3.7
4.1
3.9
3.8
4.1
4.2
3.8
3.6
4.7

Fig 7.6

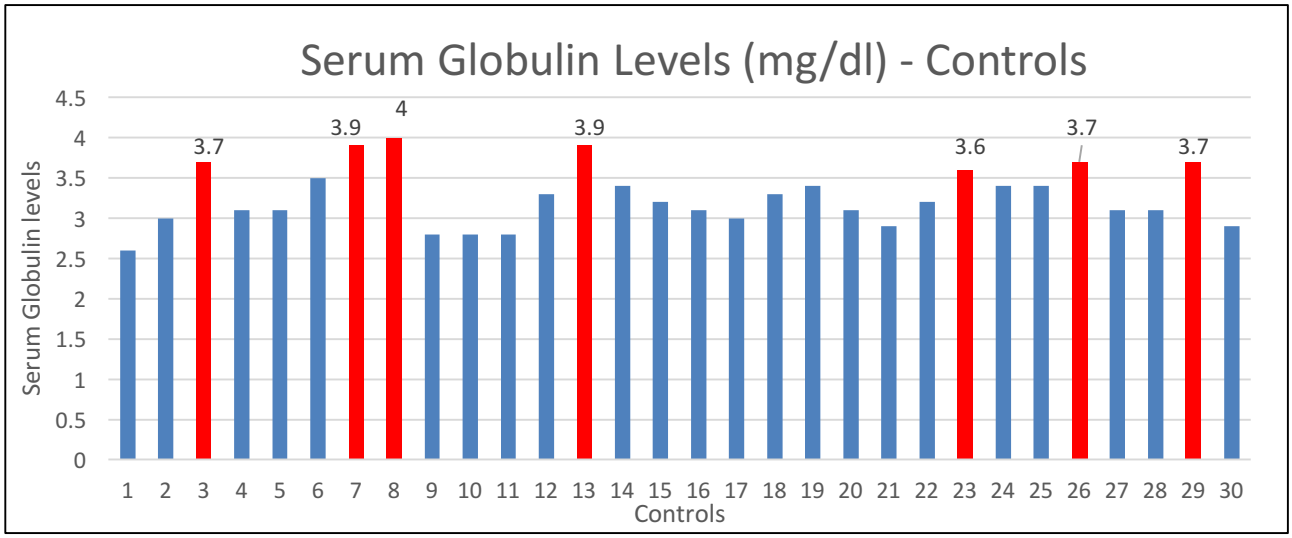
Serum Globulin Levels (mg/dl) - Controls
Serum Globulin levels
Controls
3.7
3.9
4
3.9
3.6
3.7
3.7

Fig 7.7

HYPERPROLACTINEMIA

None of the patients in the case or control group were lactating at the time of recruitment. Three patients in the case group had serum prolactin level > 25 ng/ml. Among the control population, one patient had elevated serum prolactin level. Two sample Wilcoxon rank-sum (Mann-whitney) test was used for analysis which did not show any statistically significant difference between cases and controls.

Variable	Case [Median (IQR)]	Control [Median (IQR)]	p value
Serum Prolactin level	9.06 (6.80, 15.50)	7.40 (5.40,10.20)	0.1086

Table 7.6

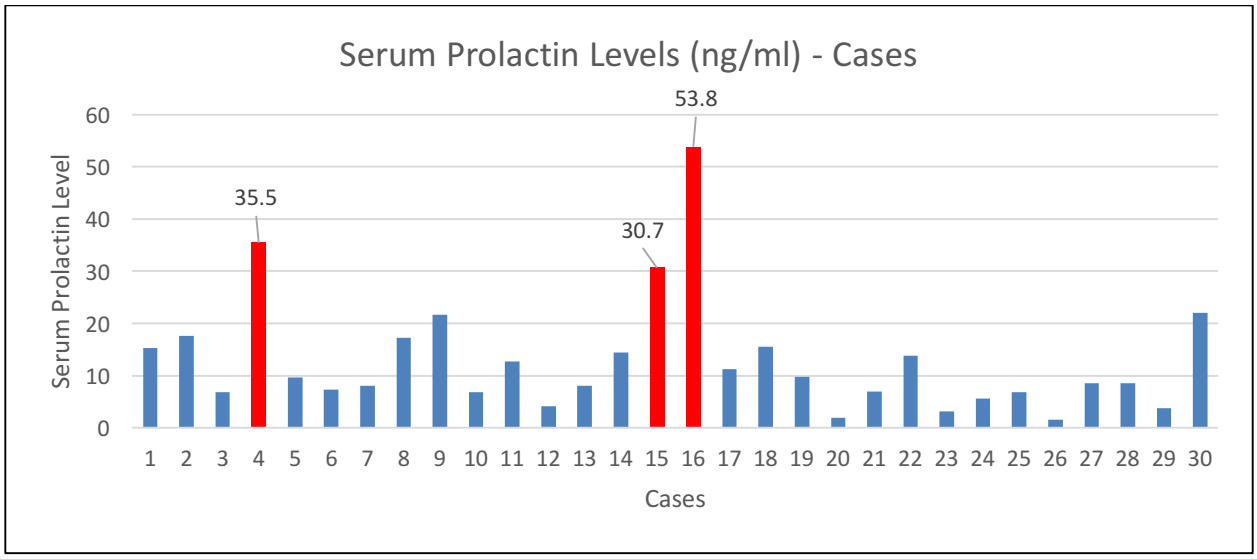
Serum Prolactin Levels (ng/ml) - Cases
Serum Prolactin Level
60
50
40
30
20
10
0
35.5
30.7
53.8
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Cases

Fig 7.8

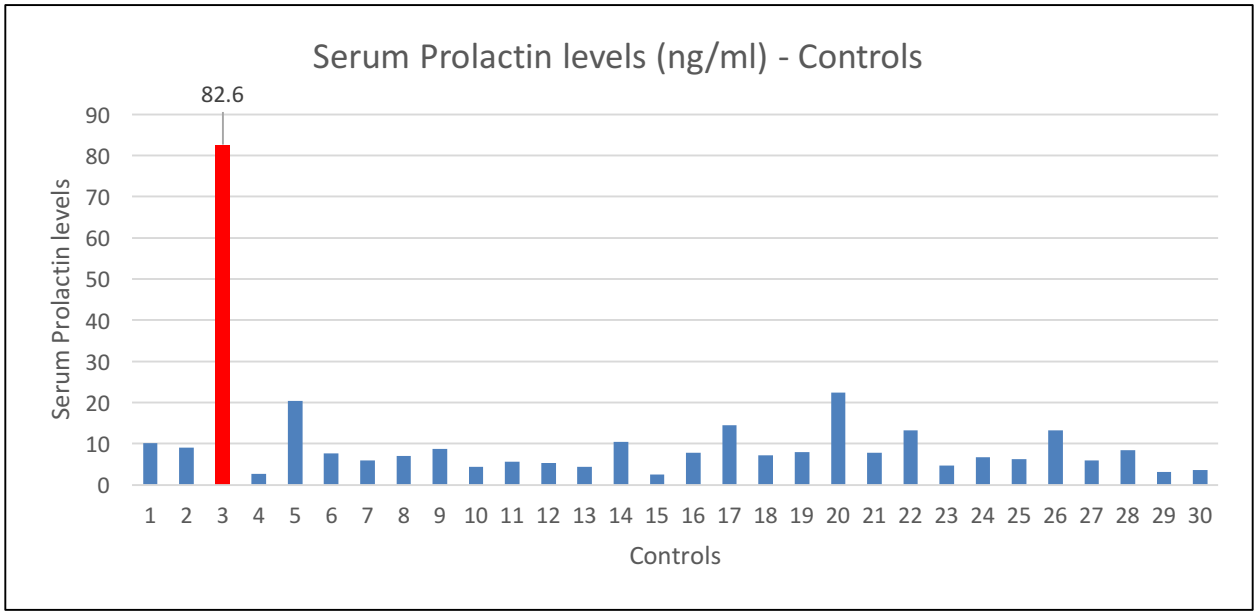
Serum Prolactin levels (ng/ml) - Controls
Serum Prolactin levels
90
80
70
60
50
40
30
20
10
0
82.6
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Controls

Fig 7.9

ANALYSIS OF OTHER RISK FACTORS

SOCIOECONOMIC STATUS

Majority of patients in both the case and control group belonged to the upper and upper-middle socio-economic scale (Modified Kuppusamy scale)(61). There was no statistically significant relationship between socio-economic status and disease (Pearson Chi^2 p value – 0.891)

SES	Cases	Controls
Upper	12 (40%)	12 (40%)
Upper middle	10 (33.33%)	8 (26.67%)
Lower middle	4 (13.3%)	4 (13.3%)
Upper lower	4 (13.3%)	6 (20%)
Lower	0	0

Pearson Chi^2 p value – 0.891

Table 7.7

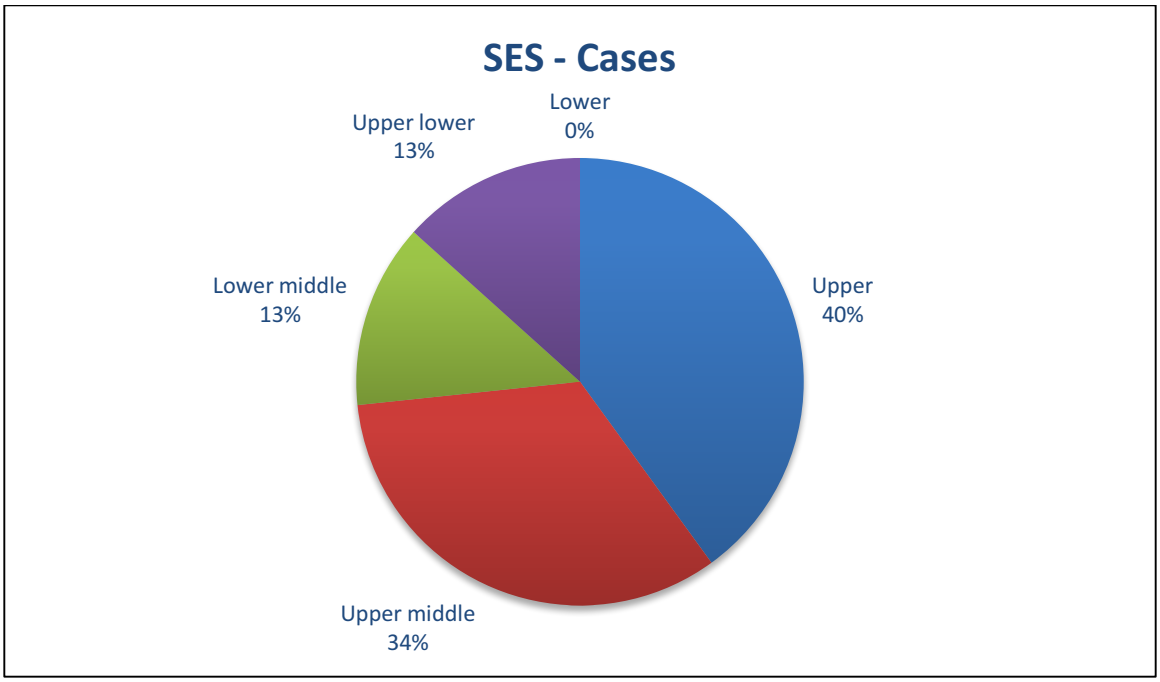

Fig 7.10

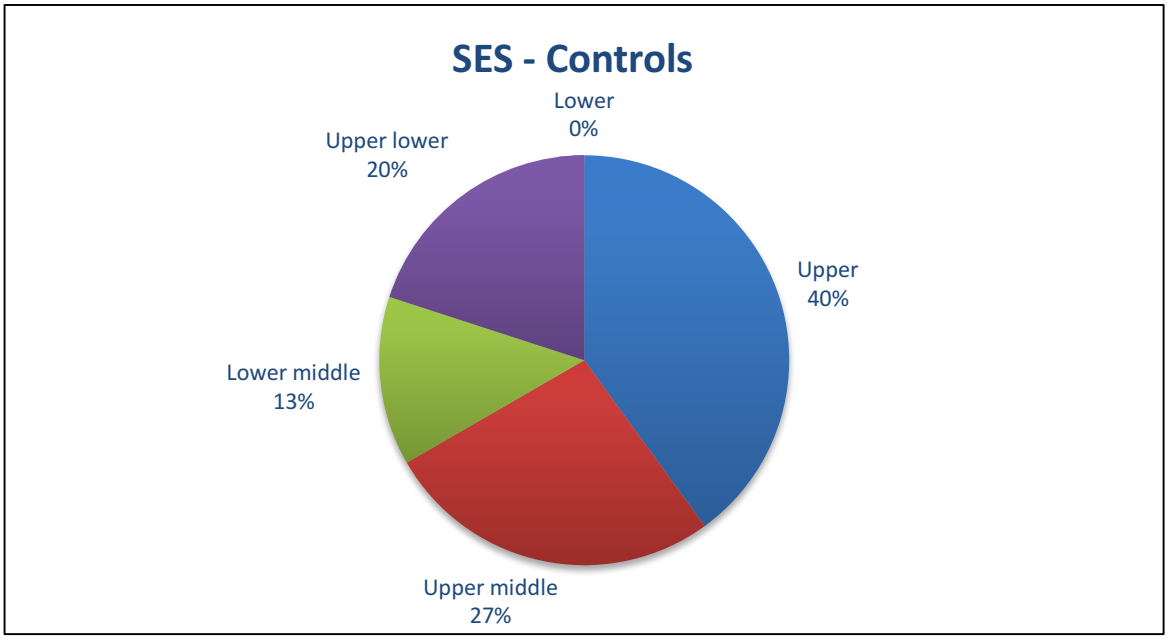

Fig 7.11

BREASTFEEDING PRACTICES

Patients in the case group had breastfed at least one child (Range 1-4, Mean 1.6) whereas one patient in the control group did not breastfeed both her children. There was no difference between cases and controls, in terms of number of children breastfed.

Variable	Case (Mean +/- S.D)	Control (Mean +/- S.D)	p value
Number of children breastfed	1.6 +/- 0.77	1.7 +/- 0.83	0.6319
Average duration of breast feeding (All lactations)	25.74 +/- 11.71	15.26 +/- 8.37	0.0002
Average duration of last breast feeding	25.16 +/- 11.62	15.44 +/- 8.62	0.0006

Table 7.8

All cases had breastfed in the past (All lactations) for an average duration of 25.74 months (Range 5-60 months). The mean duration of last breastfeeding amongst cases was 25.16 months. On the other hand, patients belonging to the control group had breastfed for an average duration of 15.27 months (Range 3-39 months) with mean duration of last breastfeeding being 15.45 months. This seemed to be a statistically significant difference between cases and controls (Two sample t-test p value = 0.0002; 0.0006)

None of the cases or controls were currently breast feeding. Average duration since last breastfeeding was 52.8 months in cases as compared to 67.9 months in controls.

66.6% of cases and 75.8% of controls always breastfed adequately. Equal number of cases breastfed on demand (50%) and periodically (50%). Amongst controls, the periodicity of breast feeding was similar (46.4% vs 53.6%). There was no difference between cases and controls in terms of adequacy and periodicity of breastfeeding.

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Adequacy of breastfeeding	Always	20 (66.67%)	22 (75.86%)	0.144
	Almost always	5 (16.67%)	7 (24.14%)	
	Occasionally	3 (10%)	0	
	Never	2 (6.67%)	0	
Periodicity of breastfeeding	On demand	15 (50%)	13(46.43%)	0.786
	Periodical	15 (50%)	15 (53.57%)	
	Every 2 hours	12 (80%)	12 (75%(	0.137
	5-10 times/day	3 (20%)	1 (6.25%)	
	< 5 times/day	0	3 (18.75%)	

Table 7.9

Most of the cases (90%) and controls (96%) breastfed on both sides. Amongst the cases, one patient exclusively breast fed on the left (reason unknown) and two patients exclusively breastfed on the right (Due to retracted left nipple and feeling of inadequate milk production and poor suckling by the infant on the left breast). All these three women had disease on the contralateral side. As mentioned above, one patient in the control group did not breastfeed both her children. However, there was no statistically significant difference between cases and controls in this aspect (Pearson Chi^2 p value – 0.217).

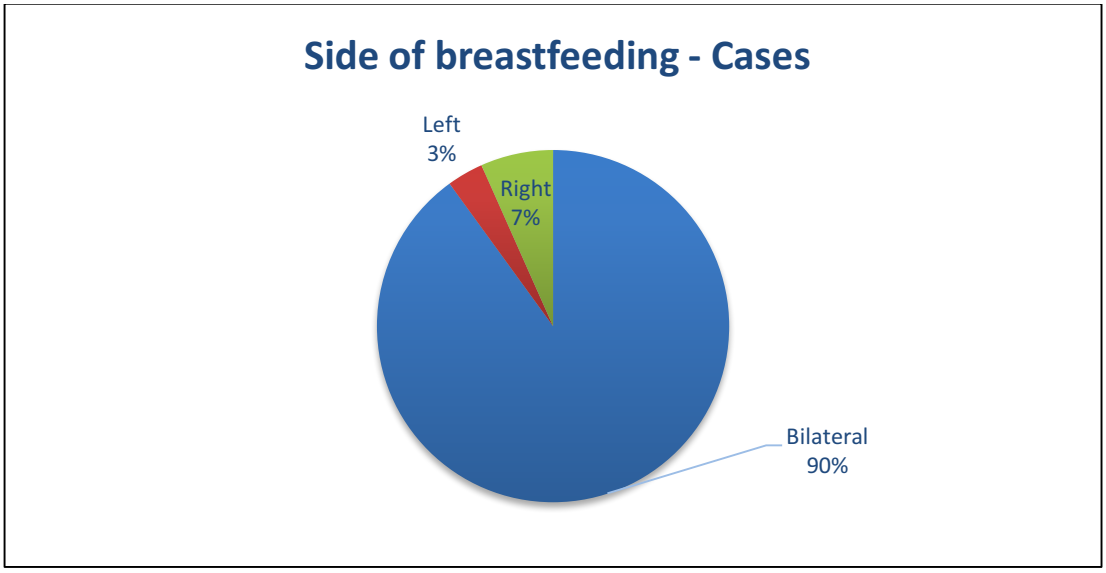

Fig 7.12

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Side of breastfeeding	Bilateral	27 (90%)	29 (100%)	0.217
	Left	1 (3.33%)	0	
	Right	2 (6.67%)	0	

Table 7.10

Use of breast pump and expression of milk was found in 20% of cases and 13.79% controls which was not statistically significant (Pearson Chi^2 p value – 0.525). Prevalence of lactation related problems such as cracked nipple, mastitis/breast abscess and breast engorgement was analyzed. Cracked nipple was reported by 13.33% of cases as compared to 6.9% in controls (Pearson Chi^2 p value – 0.413). Mastitis/breast abscess was reported by 10% of cases as compared to 6.9% in controls (Pearson Chi^2 p value – 0.669) and breast engorgement in 16.67% cases as compared to 10.34% in controls (Pearson Chi^2 p value – 0.478). There was no statistically significant difference in lactation-related problems between cases and controls.

Variable	Cases	Controls	Pearson Chi^2 p value
Use of breast pump	6 (20%)	4 (13.79%)	0.525
Use of expressed milk	6 (20%)	4 (13.79%)	0.525
Cracked nipple	4 (13.33%)	2 (6.9%)	0.413
Mastitis/Breast abscess	3 (10%)	2 (6.9%)	0.669
Breast engorgement	5 (16.67%)	3 (10.34%)	0.478

Table 7.11

43.33% of women in the case group washed hands before breastfeeding as compared to 72.41% among the control group. This difference was found to be statistically significant (Pearson Chi^2 p value – 0.024). 60% of women in the case group cleaned the breast before breastfeeding compared to 72.41% of women in the control group. However, this difference was not statistically significant (Pearson Chi^2 p value – 0.314).

Variable	Cases	Controls	Pearson Chi^2 p value
Washing hands before breastfeeding	13 (43.33%)	21 (72.41%)	0.024
Cleaning breast before breastfeeding	18 (60%)	21 (72.41%)	0.314

Table 7.12

CLOTHING HABITS

Only two patients each in the case and control group reported non-usage of brassiere (6.67%) which did not seem to have a significant difference between cases and controls. 93.33% of patients in both the groups did not have history of any allergic reaction to clothing.

Source of water for washing clothes was from borewell in 46.67% (60% in controls) and Public water supply in another 46.67% (33.33% in controls) of cases. (Pearson Chi^2 p value – 0.76)

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Usage of Brassiere	Daily	20 (66.67%)	24 (80%)	0.580
	Only outside the house	7 (23.33%)	3 (10%)	
	Occasionally	1 (3.33%)	1 (3.33%)	
	Never	2 (6.67%)	2 (6.67%)	
Hours of usage	Always	6 (21.43%)	10 (35.71%)	0.621
	< 6 Hrs	6 (21.43%)	4 (14.29%)	
	6-12 Hrs	2 (7.14%)	1 (3.57%)	
	>12 Hrs	14 (50%)	13 (46.43%)	
Frequency of change	Daily	26 (96.30%)	28 (100%)	0.304
	Once in 2-3 days	1 (3.70%)	0	
Fitting	Loose	3 (10%)	1 (3.57%)	0.337
	Fitting	24 (85.71%)	27 (96.43%)	
	Tight	1 (3.57%)	0	
Allergic reactions to clothing		2 (6.67%)	2 (6.67%)	1.00

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Source of water for washing	Borewell	14 (46.67%)	18 (60%)	0.761
	Public water supply	14 (46.67%)	10 (33.33%)	
	Reservoir	1 (3.33%)	1 (3.33%)	
	River	1 (3.33%)	1 (3.33%)	

Table 7.13

LOCAL APPLICATION OF SUBSTANCES

There was history of regular application of substances such as talcum powder, oil, moisturizer, deodorant/perfume over the breast in 36.67% of cases as compared to 20% in controls. (Pearson Chi^2 p value – 0.152). Only one patient in the case group reported allergic reaction following usage of deodorant.

TRAUMA

Three patients gave history of trauma to the breast in the case group. There was no history of trauma to the breast in any of the controls. This difference was not statistically significant. (Pearson Chi^2 p value – 0.076).

Variable	Cases	Controls	Pearson Chi^2 p value
Local application	11 (36.67%)	6 (20%)	0.152
Trauma	3 (10%)	0	0.076

Table 7.14

DIETARY HABITS

Both case and control groups predominantly consumed non-vegetarian diet. Predominant type of meat consumed was chicken in 78.57% of cases and 57.14% controls (Pearson Chi^2 p value – 0.053) with 60.7% patients (cases and controls) consuming meat less than twice a week.

55% patients (50% cases, 60% controls) never consumed canned/preserved food and 36.67% (40% cases, 33.33% controls) reported occasional consumption.

Milk consumption on a daily basis was reported in 43.33% of cases and 60% among controls whereas frequency of egg consumption was less than twice a week in 56.67% cases and 30% controls (Pearson Chi^2 p value – 0.207).

Most common oil used was Sunflower oil in 53.33% cases and 56.67% controls with no difference.

Most common source of drinking water was treated water in 46.67% cases (33.33% in controls) which was not statistically significant (Pearson Chi^2 p value – 0.293).

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Diet pattern	Vegetarian	2 (6.67%)	2 (6.67%)	1.00
	Non-vegetarian	28 (93.33%)	28 (93.33%)	
Meat consumption	Daily	5 (17.86%)	3 (10.71%)	0.791
	Alternate days	6 (21.43%)	5 (17.86%)	
	< twice a week	16 (57.14%)	18 (64.29%)	
	Occasional	1 (3.57%)	2 (7.14%)	
Predominant type of meat	Chicken	22 (78.57%)	16 (57.14%)	
	Mutton	0	6 (21.43%)	0.791
	Pork	1 (3.57%)	0	
	Beef	1 (3.57%)	0	
	Fish	4 (14.29%)	6 (21.43%)	

Milk consumption	Daily	13 (43.33%)	18 (60%)	0.294
	Alternate days	2 (6.67%)	2 (6.67%)	
	< twice a week	4 (13.33%)	1 (3.33%)	
	Occasional	5 (16.67%)	7 (23.33%)	
	Never	6 (20%)	2 (6.67%)	
Canned/Preserved food	Daily	1 (3.33%)	0	0.594
	Alternate days	1 (3.33%)	0	
	< twice a week	1 (3.33%)	2 (6.67%)	
	Occasional	12 (40%)	10 (33.33%)	
	Never	15 (50%)	18 (60%)	
Egg consumption	Daily	2 (6.67%)	1 (3.3%)	0.207
	Alternate days	4 (13.33%)	10 (33.33%)	
	< twice a week	17 (56.67%)	9 (30%)	
	Occasional	5 (16.67%)	7 (23.33%)	
	Never	2 (6.67%)	3 (10%)	
Type of oil	Coconut oil	2 (6.67%)	2 (6.67%)	0.320
	Sunflower oil	16	17 (56.67%)	

		(53.3%)		
	Mustard oil	10 (33.33%)	6 (20%)	
	Vegetable oil	1 (3.33)	0	
	Others	1 (3.33)	5 (16.67%)	
Source of drinking water	Borewell	6 (20%)	12 (40%)	0.293
	Public water supply	9 (30%)	8 (26.67%)	
	River	1 (3.33%)	0	
	Treated water	14 (46.67%)	10 (33.33%)	
Use of boiled water	Always	10 (33.33%)	11 (36.67%)	0.884
	Occasionally	3 (10%)	2 (6.67%)	
	Never	17 (56.67%)	17 (56.67%)	

Table 7.15

HYGIENE

There was no significant difference in bathing habits of cases and controls. 70% cases and 66.67% controls bathed daily where as 26.67% cases and 33.33% controls bathed twice daily. Source of bathing water was from public water supply in 50% of cases (36.67% in controls) and

borewell in 43.3% of cases (60% controls). There was no statistically significant difference between cases and controls (Pearson Chi^2 p value – 0.49).

All patients used soap regularly and only one patient in the control group reported allergic reaction to soap. Frequency of hand washing was found to be similar in both case and control group (More than 5 times daily in 76.67% of cases vs 73.33% controls).

Majority of cases (83.33%) and controls (70%) had a closed drainage system at home with no statistically significant difference. Household waste was mostly disposed safely by municipal waste collection (66.67% cases vs 63.33% controls).

With regards to menstrual hygiene, majority used pads in both groups (92.59% in cases and 100% in controls) with frequent change and no reuse.

Dryness of skin was reported by 5 cases as compared to only 2 controls. However, this difference was not statistically significant (Pearson Chi^2 p value – 0.228). Similarly, excessive sweating was reported by 7 cases as compared to 2 controls. This was also found to be statistically insignificant (Pearson Chi^2 p value – 0.071). There was no history of frequent skin infections/lesions among the case or control group.

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Source of bathing water	Borewell	13 (43.33%)	18 (60%)	0.490
	Public water supply	15 (50%)	11 (36.67%)	
	Reservoir	1 (3.33%)	0	

	River	1 (3.33%)	1 (3.33%)	
Frequency of bathing	Twice daily	8 (26.67%)	10 (3.33%)	0.536
	Daily	21 (70%)	20 (66.67%)	
	Once in 2-3 days	1 (3.33%)	0	
Frequency of hand washing	> 5 times/day	23 (76.67%)	22 (73.33%)	0.952
	2-5 times/day	6 (20%)	7 (23.33%)	
	< 2 times/day	1 (3.33%)	1 (3.33%)	
Drainage	Open	5 (16.67%)	9 (30%)	0.222
	Closed	25 (83.33%)	21 (70%	
Waste disposal	Outside the house	4 (13.33%)	5 (16.67%)	0.934
	Open ground	6 (20%)	6 (20%)	
	Municipal collection	20 (66.67%)	19 (63.33%)	

Table 7.16

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Usage of sanitary napkin/cloth	Sanitary napkin	25 (92.59%)	26 (100%)	0.157
	Cloth	2 (7.41%)	0	

Frequency of change of sanitary napkin/cloth	Many times/day	25 (92.59%)	25 (96.15%)	0.225
	Once daily	2 (7.41%)	0	
	Once in 2-3 days	0	1 (3.85%)	
Frequency of washing external genitalia	Many times/day	28 (96.55%)	28 (96.55%)	1.00
	Once daily	1 (3.33%)	1 (3.33%)	
Dryness of skin		5 (16.67%)	2 (6.67%)	0.228
Excessive sweating		7 (23.33%)	2 (6.67%)	0.071

Table 7.17

BENIGN BREAST DISEASE COMPLAINTS

The prevalence of mastalgia was found to be similar in the case group (23.33%) as compared to the control group (26.67%). 20% of cases gave history of breast lumps in the past as compared to 10% of patients in the control group. However, this difference was not statistically significant (Pearson Chi^2 p value – 0.278).

History of nipple discharge was found in 13.33% of cases with no patients in the control group having these complaints. This was found to be statistically significant (Pearson Chi^2 p value – 0.038).

Variable	Cases	Controls	Pearson Chi^2 p value
History of mastalgia	7 (23.33%)	8 (26.67%)	0.766
History of nipple discharge	4 (13.33%)	0	0.038
History of breast lumps	6 (20%)	3 (10%)	0.278

Table 7.18

ENVIRONMENT

Most women in the case group were predominantly in the household environment (83.33%). Majority of patients in both the groups did not have any exposure to pets or animals (86.67% cases, 83.33% controls).

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Environment	Household	25 (83.33%)	11 (36.67%)	0.000
	Work	5 (16.67%)	19 (63.33%)	
Exposure to pets/animals		4 (13.33%)	5 (16.67%)	0.718

Table 7.19

HORMONAL FACTORS

88.53% of women in the study were premenopausal (90% cases; 86.67% controls), whereas 11.67% women were postmenopausal (Three cases and four controls). While majority of women in the case or control group did not use OCPs on a regular basis, regular use of OCPs was reported by 7 patients in the case group and 1 patient in the control group. This difference was not statistically significant (Pearson Chi^2 p value – 0.058).

None of the patients had history of use of hormonal IUCDs. However, 3 patients in the case group gave history of hormonal therapy for infertility as compared to none in the control group.

This difference was not statistically significant (Pearson Chi^2 p value – 0.076).

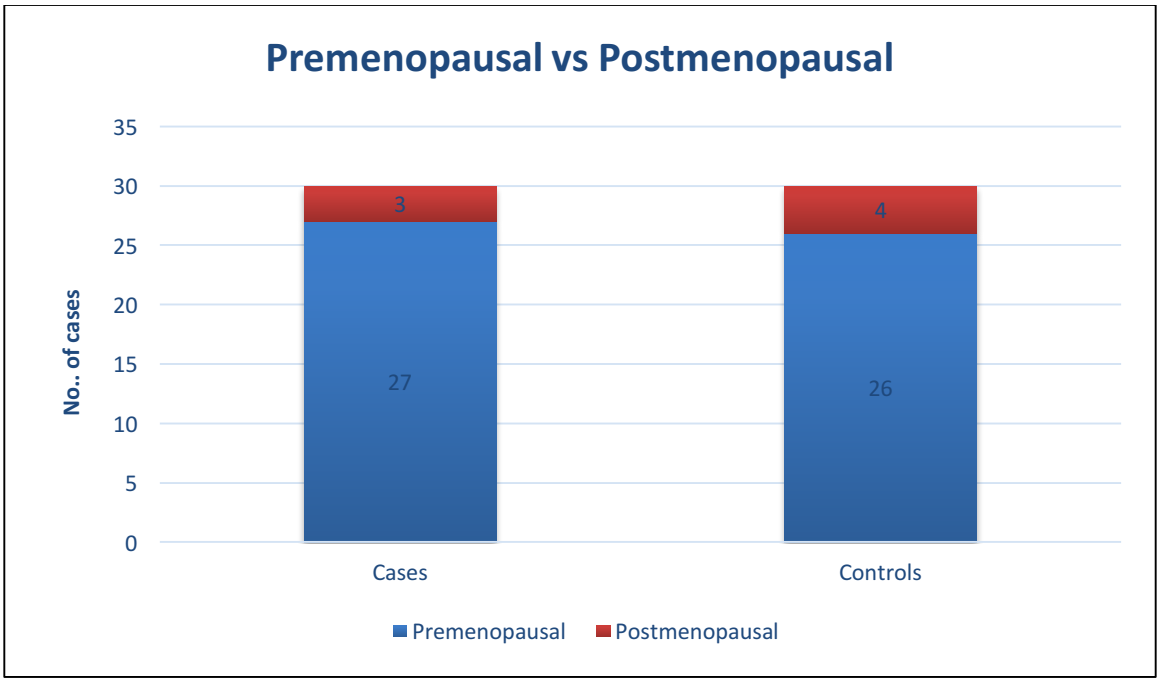

Fig 7.13

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
Regular usage of OCPs	Daily	4 (13.33%)	0	0.058
	Occasionally	3 (10%)	1 (3.33%)	
	Never	23 (76.67%)	29 (96.67%)	
Hormonal therapy for infertility		3 (10%)	0	0.076
Regular steroid use		2 (6.67%)	0	0.150
History of breast lumps		6 (20%)	3 (10%)	0.278

Table 7.20

HABITS

None of the participants gave history of smoking, consumption of alcohol or tobacco.

PREGNANCY

Parity among cases varied from 1-4 with a mean of 2.13. Among controls, parity ranged from 1-5, with a mean of 2.1. Two-sample t test did not show statistically significant difference between the means of the two groups (Two sample t-test p value = 0.8922).

Variable	Case (Mean +/- S.D)	Control (Mean +/- S.D)	p value
Parity	2.133 +/- 0.94	2.1 +/- 0.96	0.8922

Table 7.21

The average spacing between births among cases was 48 months as compared to 44.35 months in controls.

96.67% of patients received immunization in pregnancy in the case group as compared to 90% in the control group. 23.33% of patients were diagnosed with a medical comorbidity during pregnancy as compared to 13.33% in the control population. However, these observations did not show any statistically significant difference (Pearson Chi^2 p value – 0.301, 0.317). None of the cases or controls received any postnatal immunization.

Variable	Cases	Controls	Pearson Chi^2 p value
Immunization in pregnancy	29 (96.67%)	27 (90%)	0.301
Comorbidities in pregnancy	7 (23.33%)	4 (13.33%)	0.317

Table 7.22

COMORBIDITIES

5 patients among cases and 3 patients among controls were known diabetics. 2 patients were diagnosed with TB in the past in the case group as compared to none in the control group. 2 patients in the case group also gave history of contact with TB as compared to none in the control group. These differences were not statistically significant (Pearson Chi^2 p value – 0.448, 0.150, 0.150 respectively). There was no history of immunosuppressive disease among both cases and controls. Immunization status was complete in 36.67% of cases (56.6% controls) whereas 63.33% of cases and 36.67% of controls were unaware of their immunization status.

Variable	Cases	Controls	Pearson Chi^2 p value
DM	5 (16.67%)	3 (10%)	0.448
TB	2 (6.67%)	0	0.150
Contact with TB	2 (6.67%)	0	0.150

Table 7.23

Variable	Subgroup	Cases	Controls	Pearson Chi^2 p value
BCG vaccination	Yes	13 (43.33%)	18 (60%)	0.099
	No	0	2 (6.67%)	
	Unaware	17 (56.67%)	10 (33.3%)	
Immunization status	Yes	11 (36.67%)	17 (56.67%)	0.067
	No	0	2 (6.67%)	
	Unaware	19 (63.33%)	11 (36.67%)	

Table 7.24

AUTOIMMUNE SYMPTOMS

23.33% of patients in the case group reported symptoms of joint pains as compared to 6.67% in the control group. 10% of patients in the case group (0% controls) reported skin lesions and 3.33% of patients reported eye symptoms (0% controls). However, these differences were statistically insignificant (Pearson Chi^2 p value – 0.071, 0.076, 0.313 respectively). There was no family history of autoimmune disease in both cases and controls.

Variable	Cases	Controls	Pearson Chi^2 p value
Joint pains	7 (23.33%)	2 (6.67%)	0.071
Skin lesions	3 (10%)	0	0.076
Eye symptoms	1 (3.33%)	0	0.313

Table 7.25

CLINICAL PROFILE

Involvement of right breast (56.67%) was found to be more common than the left (43.33%). There were no patients with bilateral disease.

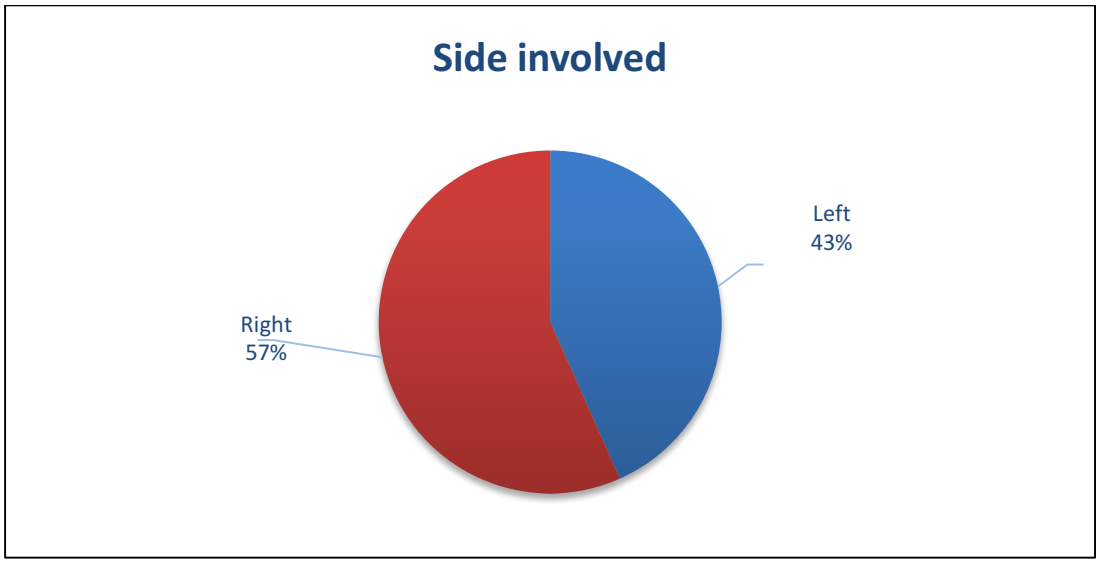

Fig 7.14

Patients mostly presented with history of breast lumps (96.67%) and pain (93.33%). 7 patients (23.3%) gave history of fever at the onset of symptoms. There was history of pus discharge in 41.38% of patients. 76.67% of patients presented with complaints of skin changes.

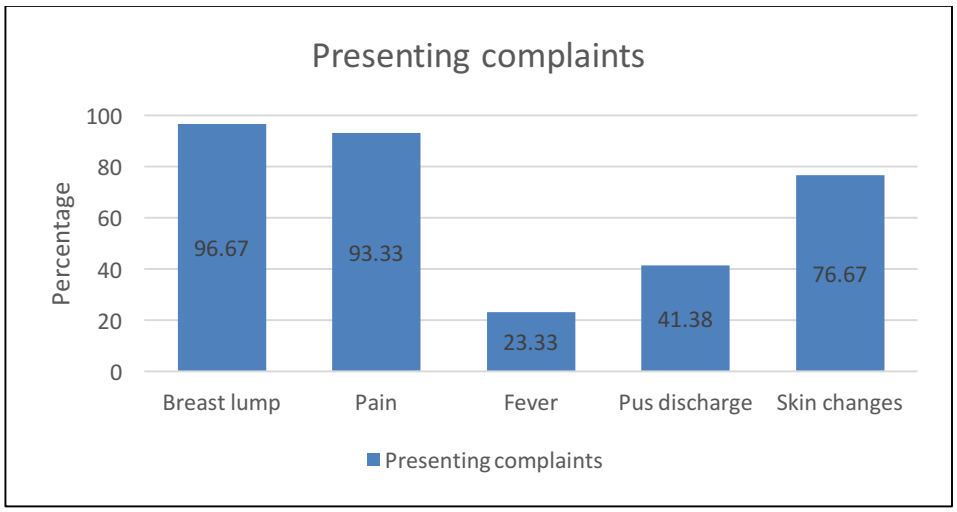

Fig 7.15

Involvement was mostly restricted to a single quadrant (66.67%) and involved multiple quadrants only in 10 patients (33.33%). Distribution amongst different quadrants are shown in the figure below.

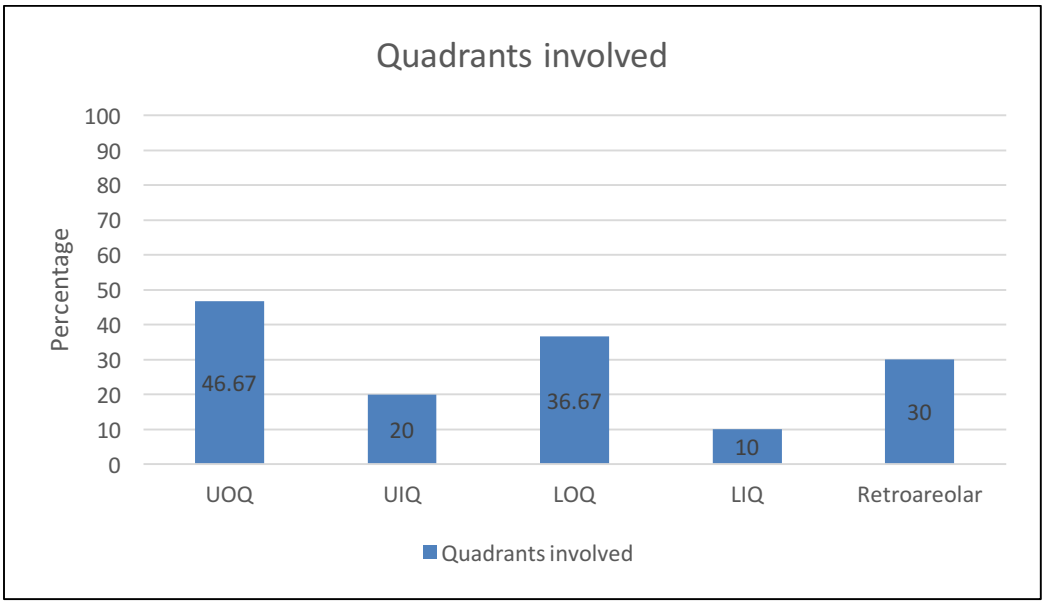

Fig 7.16

The breast lumps were on an average measuring 5.62 cm x 7.02 cm. (Dimensions ranging from 2-10 cm). 80% of breast lumps were firm in consistency with 20% being hard.

Variable	n	Mean	S.D	Min	.25	Median	.75	Max
Dimension 1	30	5.62	1.79	2.00	4.50	5.50	6.50	10
Dimension 2	30	7.02	2.26	3.00	6.00	7.00	9.50	10

Table 7.26

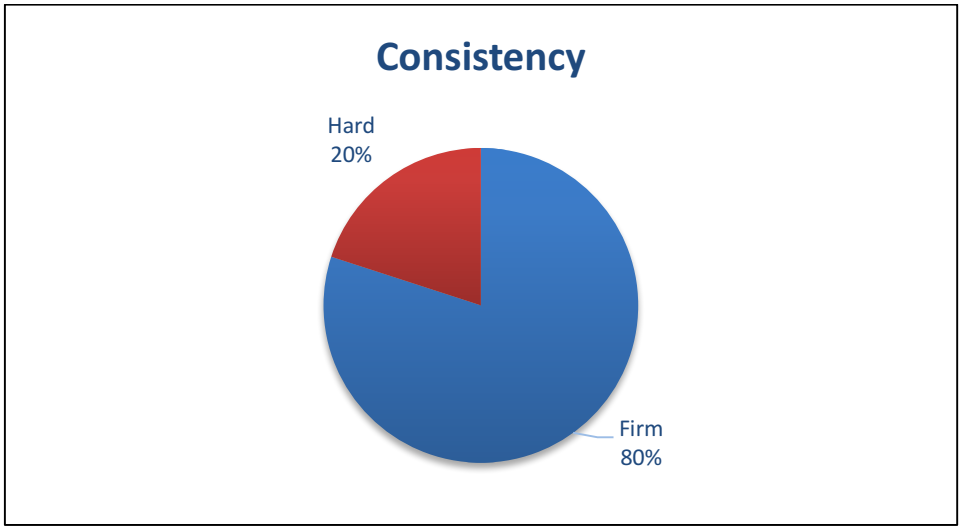

Fig 7.17

Majority of the lumps had restricted mobility as shown in the figure below

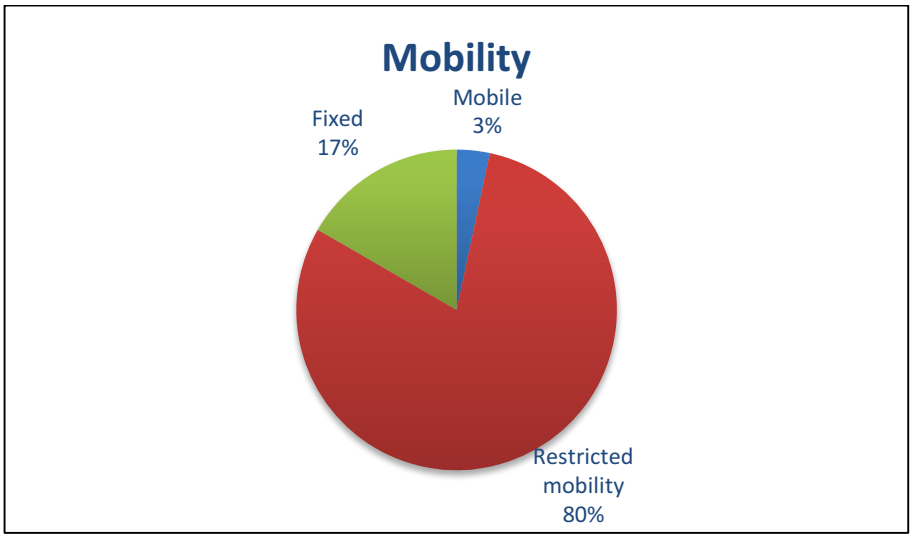

Fig 7.18

Presence of skin changes was noted in 76.67% and consisted of features such as erythema (82.61%) warmth (73.91%), skin ulceration (39.13%), discharging sinuses (36.67%), tenderness (47.83%), skin edema (34.78%) and abscess formation (39.13%). Axillary lymphadenopathy was noted in 50% of patients.

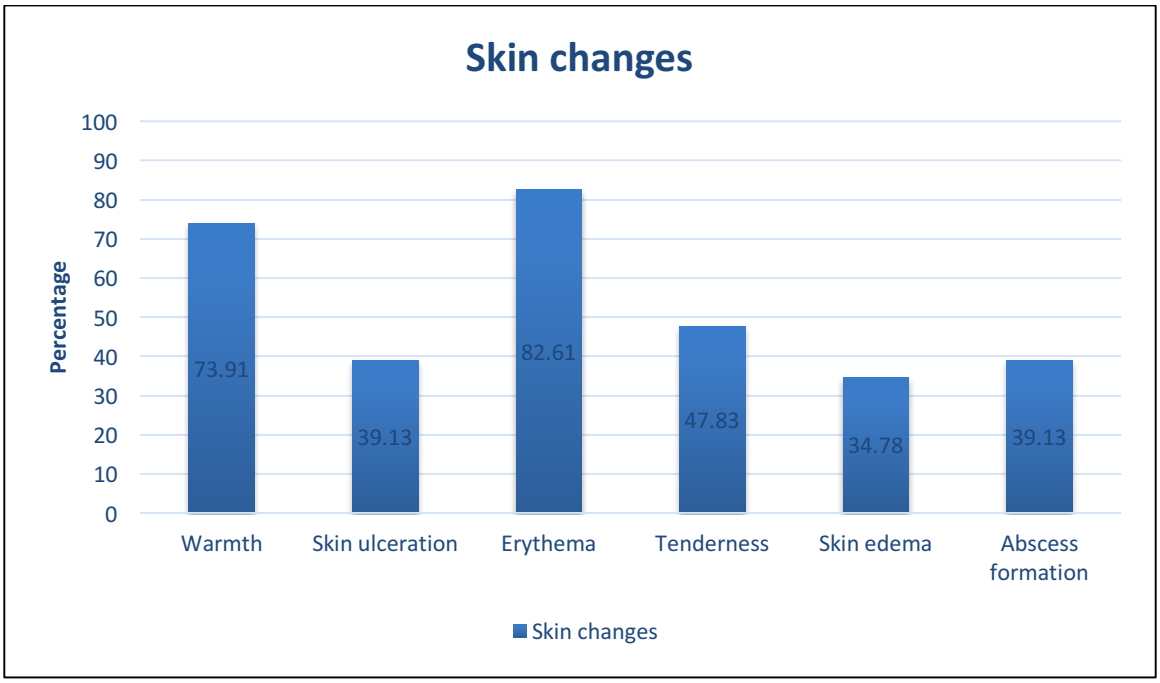

Fig 7.19

IMAGING CHARACTERISTICS

Patients underwent evaluation with Ultrasonography, Mammogram or both. Modality of imaging used has been described in the figure below.

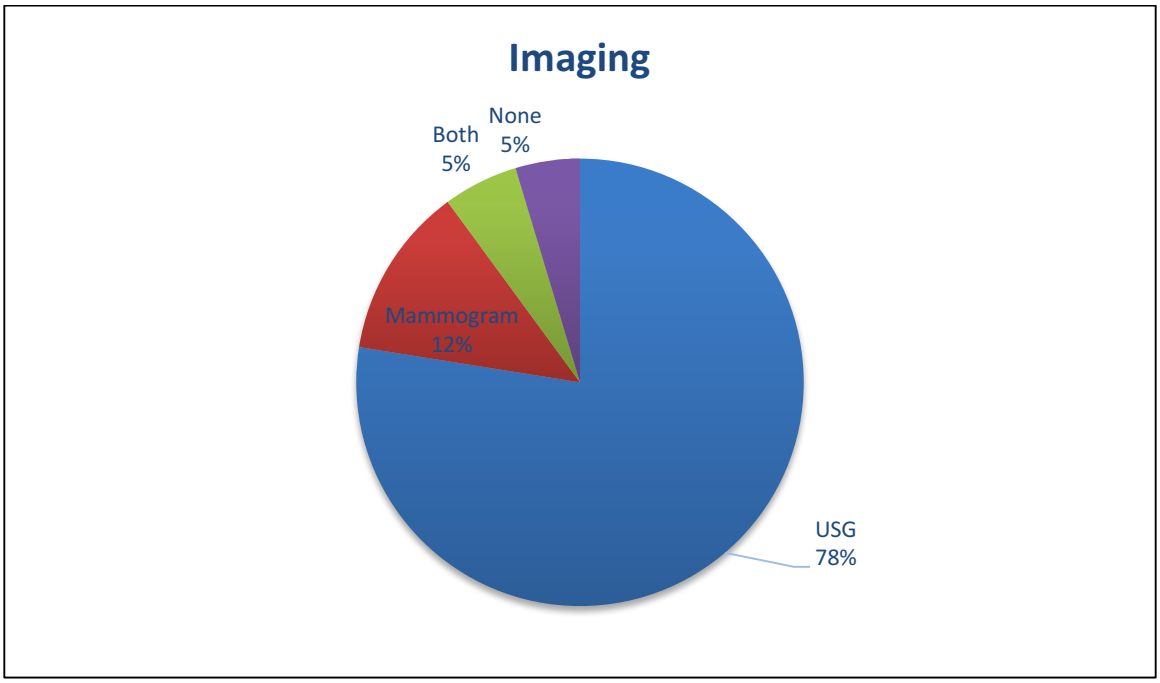

Fig 7.20

ULTRASOUND CHARACTERISTICS

The breast parenchyma in 89.47% patients was found to be predominantly fibroglandular whereas 10.53% had heterogeneous parenchyma. 9 patients (39.13%) had a discrete lesion which was mostly ill-defined (77.78%) and hypoechoic (55.56%).

52.17% of patients had evidence of collection which was hypoechoic (90.91%) with thick internal echoes (91.67%). Multiple sinus tracts were present in 47.83% of patients. There was evidence of surrounding inflammation in 47.62% and axillary lymphadenopathy was noted in 39.13%.

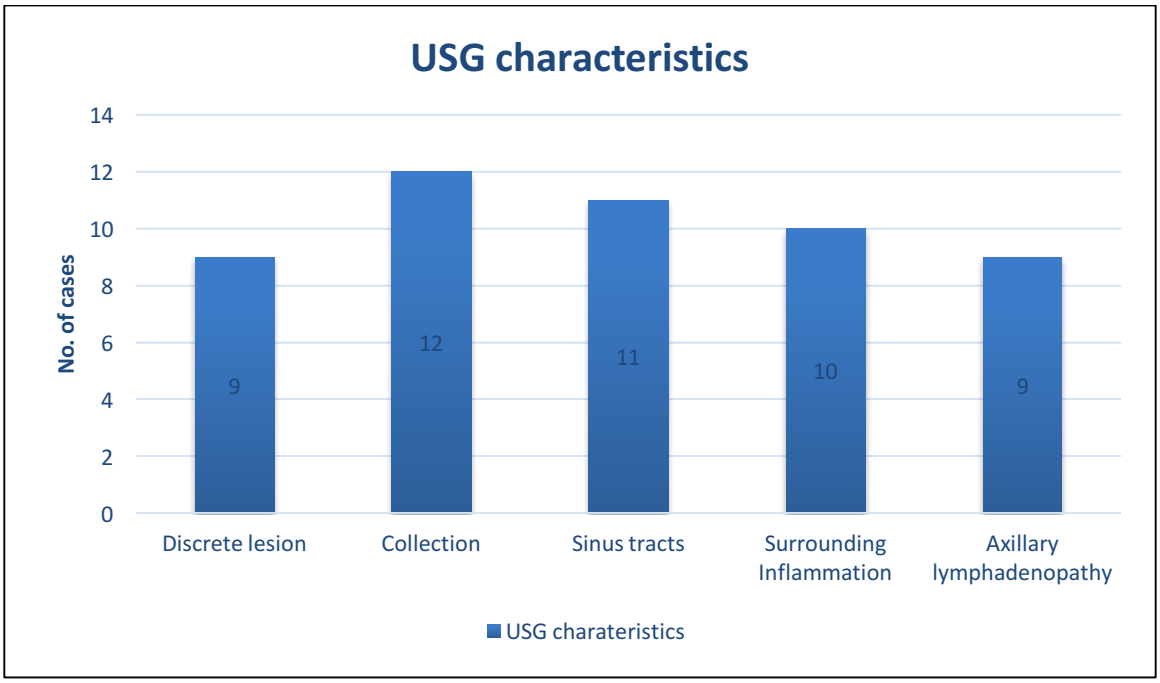

Fig 7.21

MAMMOGRAM CHARACTERISTICS

Most common parenchymal pattern was heterogeneous density (75%) with one patient having scattered areas of fibroglandular density. Only one patient had a well-defined discrete lesion and equal density. One patient had focal asymmetry (25%) and one patient had global asymmetry

(25%) with the remainder showing no asymmetry. There was no evidence of architectural distortion or calcifications.

MANAGEMENT

Patients underwent either medical, surgical or combined modality of management. One patient did not follow up for therapy.

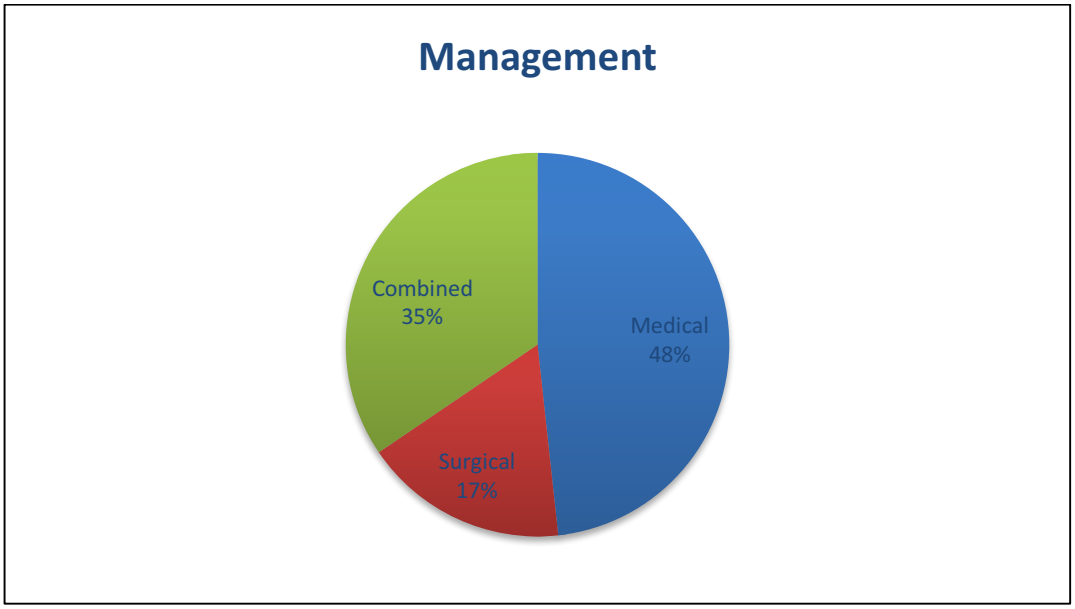

Fig 7.22

MEDICAL MANAGEMENT

Medical management was in the form of low dose steroid therapy which was given in 82.76% of patients. This was given as sole modality of management in 58.3% and preoperatively in 41.66% patients. Response to both sole steroid therapy and preoperative steroids is illustrated below

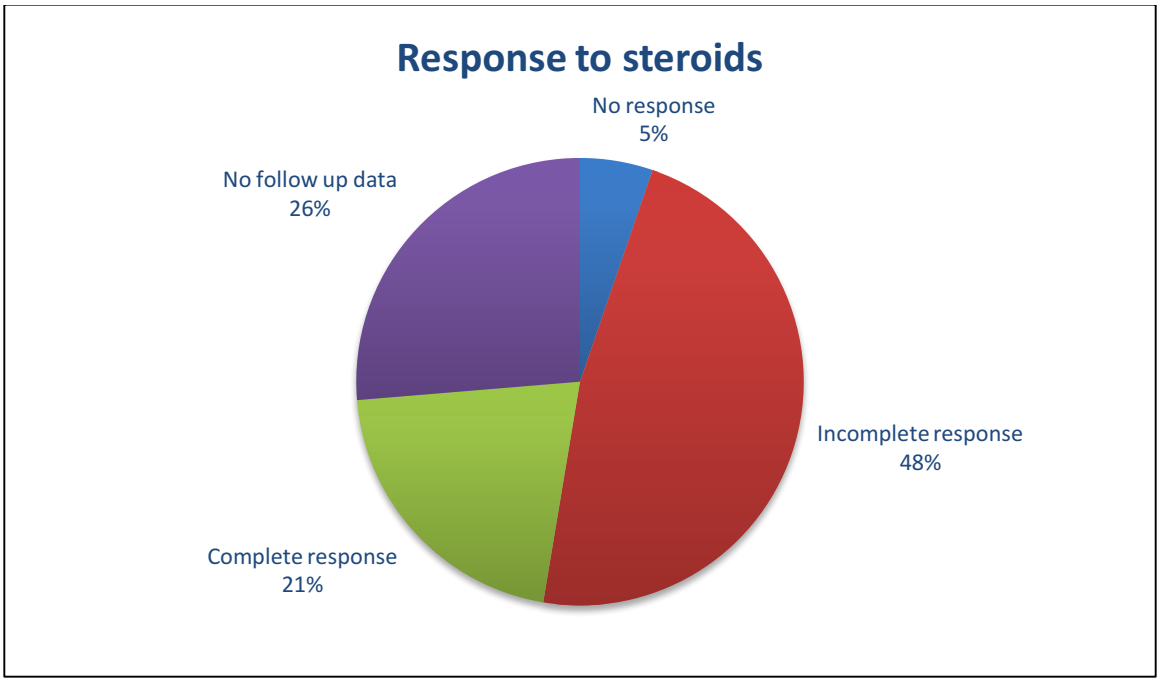

Fig 7.23

SURGICAL MANAGEMENT

Fifteen patients underwent surgical management (51.72%). Surgical management included Incision and drainage, Debridement, Excision and Mastectomy which is detailed in the figure below.

Fig 7.24 Excision

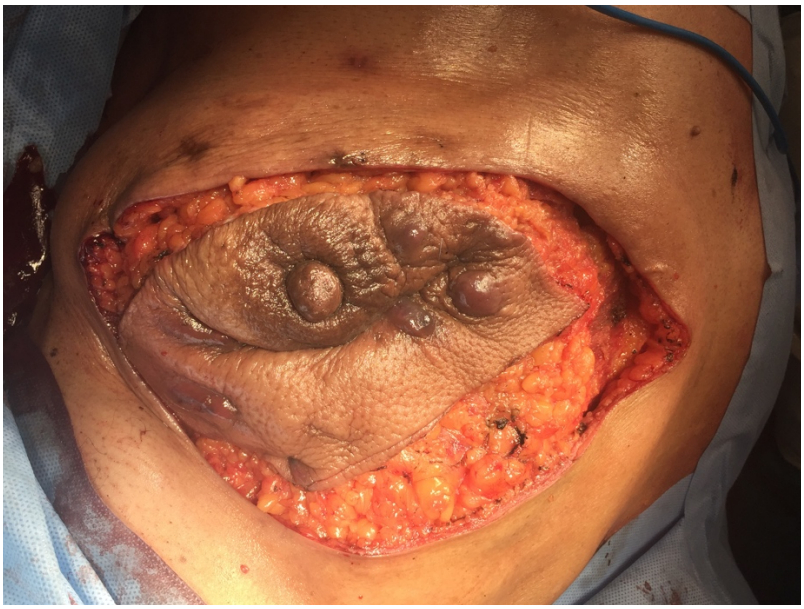

Fig 7.25 Mastectomy in a patient with IGM

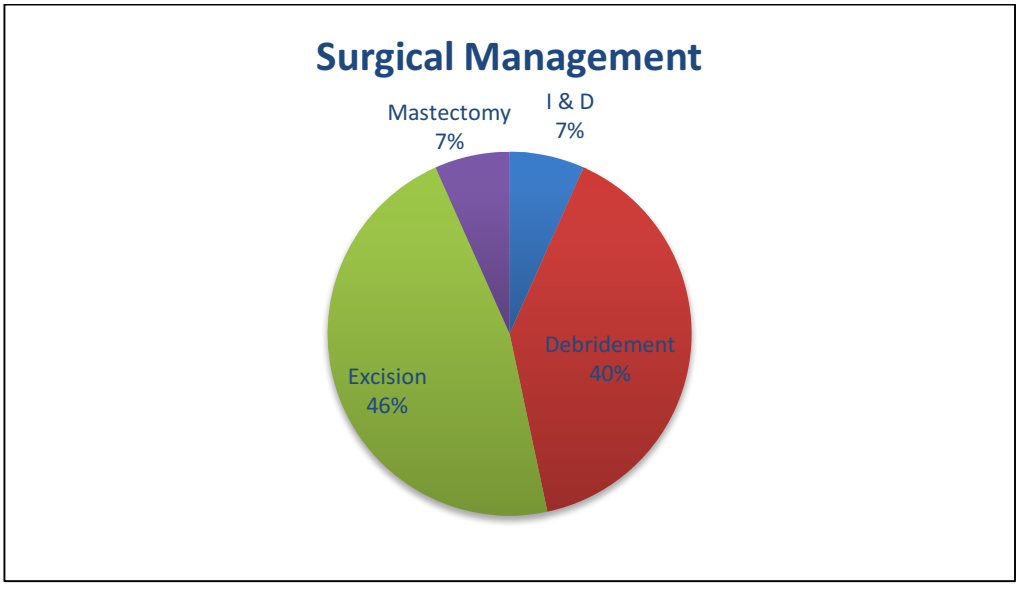

Fig 7.26

As of October 2017, fifteen patients had reviewed for follow up in OPD. There was evidence of disease progression in one patient on medical management who was planned for surgical debridement. Three patients had incomplete response to medical management with residual disease requiring re-initiation of steroid therapy. One patient showed initial response to medical management but relapsed after completing the course of steroids. Disease recurrence was noted in 2 patients, both of whom were managed solely by surgical methods. Seven patients were disease free at the time of review and one patient developed disease in the contralateral breast after having been operated on the left breast.

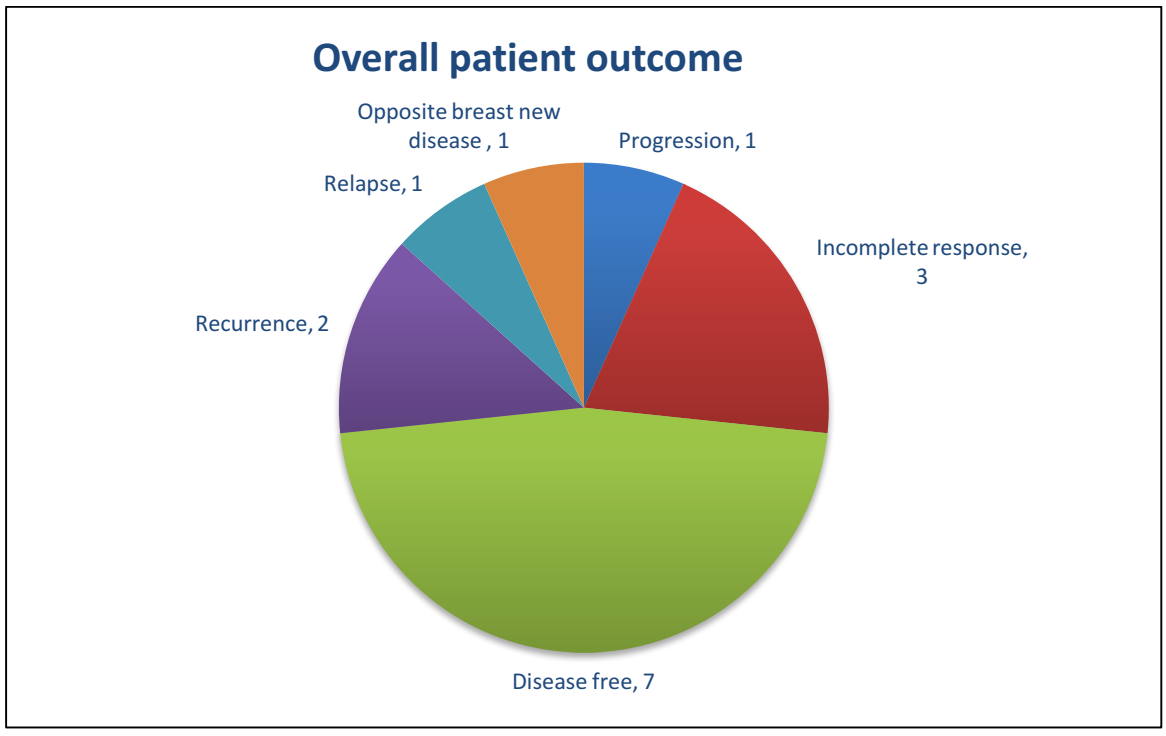

Fig 7.27

8. DISCUSSION

Granulomatous mastitis, which was earlier described as a rare chronic inflammatory disease of the breast is currently being seen more commonly in our institution. Retrospective analysis of data from our institution has shown an increasing trend over the years, as illustrated below.

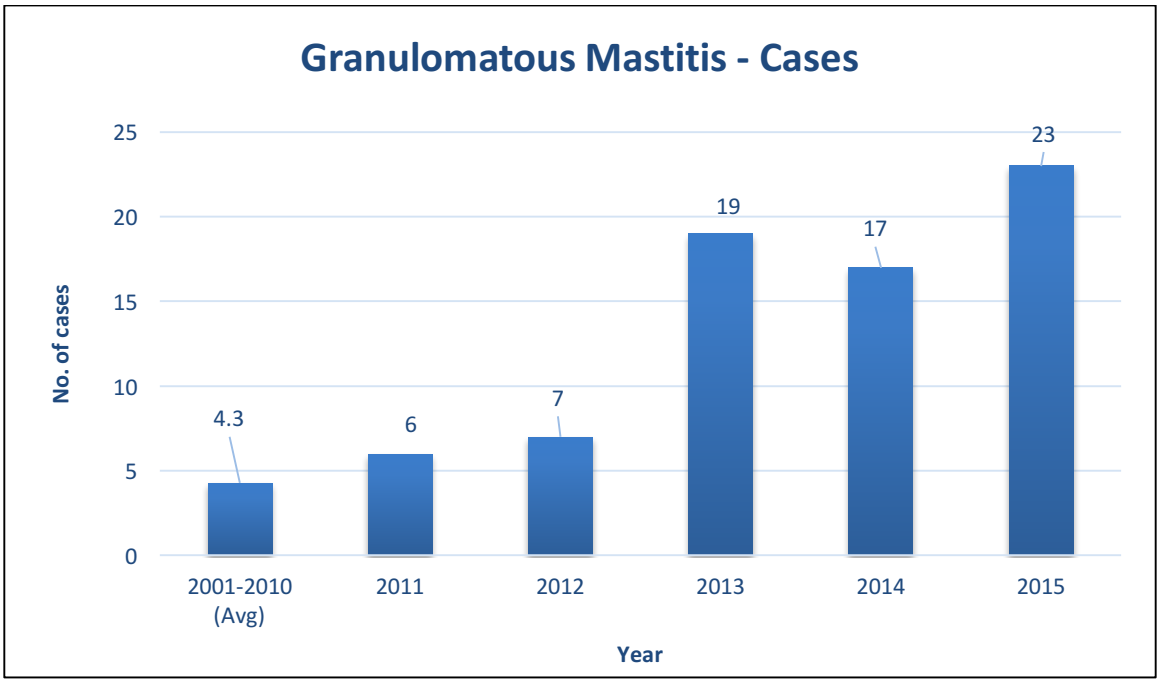

Fig 8.1

We undertook this study with an aim to elucidate the etiology of the granulomatous mastitis. As described above, we looked at the different theories that have been described in literature – Autoimmune, infectious, hormonal etiology. We also analyzed other risk factors with the help of the validated, structured questionnaire based on previously published literature and the basic pathogenesis of granulomatous inflammation.

Most of our patient population were young women with a mean age of 34 years with a wide range of 23-65 years. This was found to be similar to published literature. Unlike few published case reports and case series, all our cases were females and we did not have any male patients with granulomatous mastitis. Geographical distribution showed predominance of cases from West Bengal which is similar to the control group and reflects the larger number of patients from that region visiting our hospital every year.

The primary objective was to study the role of infectious agents and autoimmunity in the role of causation of granulomatous mastitis. In our series, Cultures were positive in only eight patients (26.7%) for a bacterial organism whereas fungal, MGIT culture and Gene XPERT TB PCR did not show any growth. The organism cultured however, were thought to be either due to contamination of the sample during sample collection, transport or inoculation (Coagulase negative staphylococcus aureus, contaminants) or superadded hospital acquired bacterial infection (MRSA). One patient in our series was treated for Tuberculous lymphadenitis and spondyloarthritis with Antitubercular therapy for 8 months and presented with a painful breast lump with sinus discharging purulent material. Core biopsy however, did not show growth of acid fast bacilli on MGIT and Gene Xpert TB PCR. She was concomitantly diagnosed with multi-drug resistant tuberculous pleural effusion and was treated with second line ATT for the same. Despite harboring Mycobacteria, tissue from the breast did not reveal the presence of organism. Thus, tuberculosis is a rare etiology for granulomatous inflammation in the breast, even in a population with high incidence.

In order to look at the role of autoimmunity, we analyzed anti-nuclear antibodies which was used as a broad marker for autoimmunity in the serum of cases as well as controls. Only one patient in our series showed positivity for serum ANA. However, this patient was already diagnosed with Rheumatoid arthritis and was on treatment for the same.

Serum Globulin levels and Immunoglobulin levels, in patients whose globulin levels were elevated were also measured. However, there was no evidence of hypergammaglobulinemia in any of the cases.

Two patients were diagnosed with arthritis and one of them had also presented with multiple skin lesions suggestive of Erythema nodosum. However, both of them were ANA negative.

Analysis of autoimmune symptoms such as history of joint pain, skin lesions, eye symptoms, prolonged fever, oral or genital ulcers and family history of autoimmune diseases was done between cases and controls. Though there was a higher number of cases than controls who gave history of joint pain, skin lesions and eye symptoms, this difference was not statistically significant. Hence, the possibility of a systemic autoimmune phenomenon causing granulomatous inflammation seems to be unlikely.

One of the secondary objectives was to look for presence of other risk factors. This was categorized into breastfeeding practices, clothing habits, application of local substances, local trauma, dietary habits, hygiene practices, addictive habits, environmental exposure, hormonal factors, pregnancy-related factors and other illness-related factors.

Most women from both groups breastfed from both sides and only three cases did not breastfeed on the diseased side. Between the two groups, there was a statistically significant difference in the duration of breastfeeding and history of washing hands before initiating breastfeeding.

Lactation-related problems such as mastitis, abscess, engorgement and cracked nipple were more among the case group, but the difference was not statistically significant.

Comparison of clothing habits, local application, local trauma, dietary habits, hygiene practices and environmental factors between cases and controls did not reveal any significant difference to suggest a role in disease causation. Pregnancy-related factors and other illnesses did not seem to have a bearing on disease causation.

Regular OCP use and history of hormonal therapy for infertility was found to be more in cases than controls which was however, was not statistically significant. None of the other hormonal factors analyzed seemed to be significantly different among the two groups.

The other secondary objective was to establish the clinical profile of patients with granulomatous mastitis. Right breast was involved more commonly than the left and disease was mostly in the upper outer quadrant, as in other breast diseases due to the presence of more breast tissue in this quadrant. The most common presentation was painful breast lumps which were firm in consistency with restricted mobility. Skin changes suggestive of inflammation were noted in majority of these cases (76.7%). Half of the patients also had palpable axillary lymphadenopathy.

There has been a paradigm shift in management of these patients from purely surgical therapy to combined therapy and even upfront medical therapy followed by reassessment and surgical intervention only in the setting of recurrence, relapse or partial response. Response to steroid therapy was noted to be varied with majority showing partial response. Three out of four patients who developed recurrence, underwent only surgical therapy with no administration of preoperative or postoperative steroids.

9. CONCLUSION

There was no evidence of uncommon infections caused by bacterial, mycobacterial or fungal pathogens. Markers for systemic autoimmunity such as Serum ANA, Immunoglobulin levels did not show any positivity amongst our cases. There was no significant hyperprolactinemia in our cases. The role of other risk factors such as breastfeeding practices, clothing habits, application of local substances, local trauma, dietary habits, hygiene practices, habitual substance usage, environmental exposure, hormonal factors, pregnancy-related factors and other illness-related factors were also not found to be significant. The etiology of Granulomatous mastitis continues to be elusive.

However, the investigators did recognize the possibility of a localized autoimmune phenomenon that was not analyzed as part of this study. Hence, we propose that further studies need to be done in this regard and intend to carry out the next study to look at immunohistochemical analysis for immunological cells such as T and B lymphocytes in the biopsy specimen to investigate localized autoimmune phenomenon.

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ANNEXURE – I

PATIENT INFORMATION SHEET

ETIOLOGICAL EVALUATION OF IDIOPATHIC GRANULOMATOUS MASTITIS BY A PROSPECTIVE CASE CONTROL STUDY

DEPARTMENT OF GENERAL & ENDOCRINE SURGERY

CHRISTIAN MEDICAL COLLEGE, VELLORE

PATIENT INFORMATION SHEET

You are being invited to participate in a study to evaluate the factors that may be associated with the condition Idiopathic granulomatous mastitis. Thank you for agreeing to be part of this study.

What is the background behind this study?

Idiopathic granulomatous mastitis is a chronic inflammatory condition (Pain, swelling and redness) affecting the breasts. It commonly affects young women, usually within 5 years of childbirth and breastfeeding. The condition has a prolonged course often reappearing after treatment. Over the past few years, the number of patients presenting to CMCH, Vellore have been gradually increasing. The causative factors and mechanism of the disease is poorly understood.

What is the purpose of the study?

Among the many theories proposed, the most widely accepted mechanism is said to be increased proteinaceous secretion in the ducts of the breast due to hormonal imbalances. This causes

collection of secretions within the duct leading to perforation of the ducts and leakage into the breast tissue causing a inflammatory reaction. This reaction is believed to be an autoimmune phenomenon (reaction of the body to self). The other theories proposed are Smoking, Pregnancy, Childbirth and breastfeeding, Infection (Bacterial, TB, Fungi), Oral contraceptive pill use, Hyperprolactinemia (Increased levels of hormone prolactin involved in milk secretion), Autoimmunity (reaction of the body to self) and Ethnicity. The purpose of this study is to analyse the probable causative factors for Granulomatous mastitis and look at the demographic and clinic profile of the patients.

If you take part what will you have to do?

If you agree to participate in this study, you will be asked a few questions about your personal details (such as age, socioeconomic status, state of origin etc.,) and about your disease (such as duration, chief complaint, questions on risk factors etc.). You will be examined by the doctor and findings will be noted. We will be taking blood samples along with the routine samples using standard universal precautions for evaluation of markers to look at autoimmunity (Serum ANA, Specific autoantibodies, Globulins and Immunoglobulin levels) and Hyperprolactinemia (Serum prolactin). All other treatments that you are already on will be continued and your regular treatment protocol for the disease will not be changed due to this study.

Can you withdraw from this study after it starts?

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your usual treatment at this hospital in any way.

What will happen if you develop any study related injury?

We do not expect any untoward event to occur due to the study as there will be no risk or intervention. The blood samples will be taken along with the routine samples.

Will you have to pay for the blood test?

The blood test taken from you will be performed free of cost for the purpose of this study.

The regular diagnostic and treatment protocol (Biopsy, Routine bacterial staining and culture, AFB staining and culture, Fungal staining and culture) will be continued and the expenses will have to be met by you, according to the hospital regulations.

Will your personal details be kept confidential?

The results of this study will be published in a medical journal but you will not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission, should you decide to participate in this study.

If you have any further questions, please contact DR. , (Ph) or email:

ANNEXURE – II

INFORMED CONSENT FORM

Study Title: ETIOLOGICAL EVALUATION OF IDIOPATHIC GRANULOMATOUS MASTITIS BY A PROSPECTIVE CASE CONTROL STUDY

Study Number:

Participant's name:

Date of Birth / Age (in years):

I_____

_____, daughter of _____

(Please tick boxes) declare that

(i) I confirm that I have read and understood the information sheet dated _____ for the above study and have had the opportunity to ask questions. [ ]

(ii) I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. [ ]

(iii) I understand that the study staff, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw

from the trial. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published. [ ]

(iv) I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s). [ ]

(v) I agree to take part in the above study. [ ]

1. Signature (or Thumb impression) of the Subject/Legally Acceptable Guardian

Date: _____/_____/_____

Signatory's Name: _____

2. Signature of the Investigator: _____

Date: _____/_____/_____

Study Investigator's Name:

3. Signature or thumb impression of the Witness: _____

Date: _____/_____/_____

Name & Address of the Witness: _____

ANNEXURE – III

CLINICAL RESEARCH FORM

ETIOLOGICAL EVALUATION OF IDIOPATHIC GRANULOMATOUS MASTITIS BY A PROSPECTIVE CASE CONTROL STUDY

STRUCTURED STUDY QUESTIONNAIRE

PATIENT DEMOGRAPHICS

Study Number: Hospital Number:

Contact no. (Landline with STD code):

Contact no. (Mobile):

Name:

Address:

Age at presentation:

State of Origin:

Socioeconomic score: 1. Occupation of the Head:

2. Family Income per month:

3. Education of the head:

Total score:

Socioeconomic scale (Modified Kupusamy scale):

RISK FACTOR ANALYSIS BREASTFEEDING PRACTICES

No. of children breastfed:

Average duration of breastfeeding in all pregnancies:

Currently breastfeeding: Yes/No Duration of last breastfeeding:

Breastfed last child: Yes/No Time since last breastfed:

Adequacy of breastfeeding: Always/Almost always/ Occasionally/ Never

Frequency of breast feeding: On demand/periodically

If periodically: Every 2 hours/ 5-10 times a day/ <5 times a day

Side of Breastfeeding: Bilateral/Unilateral If unilateral: Left/Right

Reason for Unilateral breast feeding: Feeling of inadequate milk production/ Retracted nipple/ Cracked nipple/ Breast engorgement/ Breast Abscess or mastitis/Others_____

Use of breast pump/Expressed breast milk: Yes/ No

History of cracked nipple during lactation: Yes/ No If yes, Managed by: Medical/Surgical

History of Mastitis/abscesses during lactation: Yes/ No If yes, Managed by: Medical/Surgical

History of breast engorgement: Yes/ No If yes, Managed by: Medical/Surgical

Cleaning of breast prior to breastfeeding: Yes/ No

Washing hands prior to breastfeeding: Yes/No

Cleaning of breast after breastfeeding: Yes/ No

Washing hands after breastfeeding: Yes/ No

CLOTHING HABITS

Usage of brassiere: Daily/ Only outside the house/ Occasionally/ Never

Hours of usage of brassiere: Always/ <6 Hrs/ 6-12 Hrs/ >12 Hrs/ Others_____

Frequency of change of brassiere: Daily/ Once in 2-3 days/ Once a week/ Others_____

Fitting of undergarments: Loose/Fitting/Tight

Any allergic reactions to use of brassiere: Yes/ No

Source of water used for washing: Borewell/ Public water supply/ Well/ Reservoir/ River/ Polluted water/ Others_____

LOCAL APPLICATION

Regular application of talcum powder: Yes/ No

Regular application of oil: Yes/ No

Regular application of moisturiser: Yes/ No

Regular application of turmeric powder: Yes/ No

Regular use of deodorant/perfume: Yes/ No

Regular application of any other substances: Yes/ No

Any allergic reactions to application of any substances: Yes/ No

TRAUMA

Any history of trauma to the breast: Yes/ No

Duration since trauma:_____

FOOD PRACTICES

Predominant diet pattern: Vegetarian/ Non-vegetarian/ Mixed

If non-vegetarian, Type of meat: Chicken/ Mutton/ Pork/ Beef/ Fish/ Others
_____

Frequency of consumption of meat: Daily/ Alternate days/ < Twice a week/ Occasional/ Never

Predominant cereal/ pulse based diet:

Details:_____

Regular intake of canned/preserved foods/pickles: Daily/ Alternate days/ < Twice a week/ Occasional/ Never

Regular intake of milk: Daily/ Alternate days/ < Twice a week/ Occasional/ Never

Type of milk: Cow/ Buffalo/ Goat

Boiled/ Raw milk

Regular intake of eggs: Daily/ Alternate days/ < Twice a week/ Occasional/ Never

Cooked/ Raw eggs

Type of Oil:

Particular foods ingested during pregnancy/ lactation _____

Source of drinking/Cooking water: Borewell/ Public water supply/ Well/ Reservoir/ River/ Polluted water/ Others_____

Use of boiled water: Always/ Occasionally/ Never

HYGIENE AND PERSONAL HABITS

Frequency of bathing: Twice daily/ Daily/ Once in 2-3 days/ Once a week/ Others_____

Source of bathing water: Borewell/ Public water supply/ Well/ Reservoir/ River/ Polluted water/ Others_____

Usage of soap: Always/ Occasionally/ Never

Any allergic reactions to soap: Yes/ No

Frequency of hand washing: > 5 times/ 2-5 times/ <2 times

Type of drainage system: Open/ Closed

Waste disposal: Outside the house/ Open ground/ Municipal waste collection

Excessive sweating: Yes/ No

Frequent skin infections: Yes/No

Dryness of skin: Yes/ No

BENIGN BREAST COMPLAINTS

Mastalgia: Yes/ No

Nipple discharge: Yes/ No

Breast lumps: Yes/ No

HABITS

Smoking: Yes/No

Alcohol: Y/N

Tobacco: Y/N

ENVIRONMENT

Predominant work environment/Predominant household environment

Exposure to pets/ Animals: Yes/ No

MENSTRUAL AND HORMONAL FACTORS

Age at Menarche: Age at menopause:

Regular/ Irregular periods Usage of sanitary pads/ cloth

Frequency of change of pads/cloth: Many times daily/ Once daily/ Once in 2-3 days/ Once a week/ Others_____

Reuse of pads/cloth: Yes/ No

Frequency of washing external genitalia: Many times daily/ Once daily/ Once in 2-3 days/ Once a week/ Others_____

Regular oral contraceptive pill usage: Yes/ No

Frequency: Daily/ Occasionally

Type of OCP: Combination pills/ Progestin only pills/ Emergency pills/ Others_____

Hormonal IUCD use: Yes/ No Duration

Infertility treatment: Yes/ No

Steroid use: Yes/ No

Galactorrhea: Yes/ No

PREGNANCY

Parity: Para ____ Living ____ Abortions____SB____ Neonatal/childhood deaths____

Average spacing between births: Last Child birth:

Immunization during last pregnancy:

Treatment during last pregnancy:

Comorbidities during last pregnancy:

Immunisation in the postnatal period:

COMORBIDITES AND ILLNESSES

DM: Yes/ No

Immunosuppression: Yes/ No

History of previous TB: Yes/ No

History of contact with TB: Yes/ No Other comorbidities:

Duration:

Treatment history: ATT/ Immunosuppressants/Others

Recent viral illnesses: Yes/ No

BCG Vaccination: Yes/ No/ Unaware

Immunization status: Complete / Incomplete/Unaware

CLINICAL PROFILE

PRESENTING COMPLAINTS

Involved breast: Unilateral/Bilateral If Unilateral, Side involved: Left/Right

Breast Lump: Yes/ No If yes Duration:

Associated symptoms:

Pain: Yes/ No Duration:

Fever: Yes/ No Duration:

Skin changes: Yes/ No Duration:

Warmth Duration:

Tenderness Duration:

Skin ulceration Duration:

Abscess formation Duration:

Erythema Duration:

AUTOIMMUNE SYMPTOMS

Joint pains/swelling: Yes/ No

Skin Lesions: Yes/ No If yes: Photosensitivity/Skin tightening/Vasculitic skin rash/Skin ulcers

Eye symptoms: Yes/ No If yes: Dryness of eyes/Redness and watering of eyes

Prolonged fever: Yes/ No

Mucosal ulcers: Yes/ No If yes: Oral/Genital

Other relevant symptoms:

Family history of autoimmunity: Yes/ No

EXAMINATION

LOCAL EXAMINATION:

Involved breast: Unilateral/Bilateral If Unilateral, Side involved: Left/Right

Quadrant of breast involved: UO / UI / LO / LI / Retroareolar

Breast lump: Yes/ No

Size: Consistency Soft / Firm / Hard

Discharging sinus: Yes/ No If yes : Colour of discharge

Nipple discharge: Yes/ No If yes : Colour of discharge

Mobility Mobile/Fixed

Inflammatory skin changes: Yes/ No

Warmth

Tenderness

Skin edema

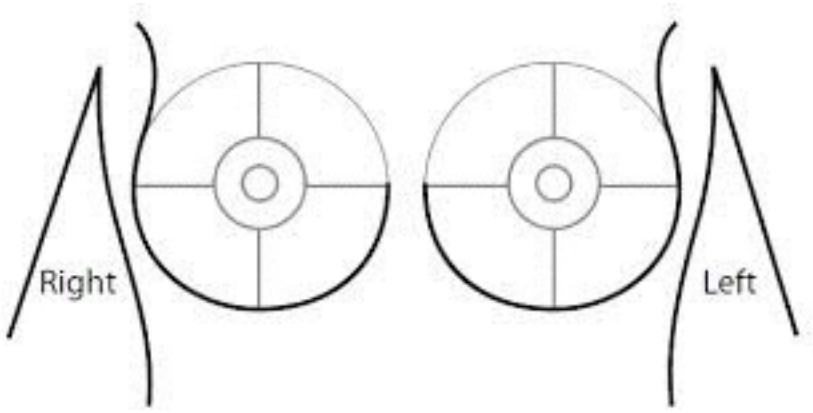
Skin ulceration

Abscess formation

Erythema

Ipsilateral Axillary lymphadenopathy : Yes/ No

PICTORICAL REPRESENTATION



LABORATORY PARAMETERS

Serum ANA: Positive/Negative

Intensity: 1+/2+/3+

Dilution:

Specific autoantibody:

Globulin levels:

Immunoglobulins: IgM IgG IgA

Serum prolactin:

Bacterial culture:

MGIT culture:

Fungal culture:

Gene XPERT TB PCR:

IMAGING

Modality: USG / Mammogram / Others

BIRADS score: 0 / 1 / 2 / 3 / 4 / 5

Quadrants involved: UO / UI / LO / LI / Retroareolar

Specific features:

MANAGEMENT

Medical/Surgical/Medical + Surgical

Pre-op/Post-op steroids Duration:

Response to steroids: Complete/Partial/No response

Surgery: I&D/Debridement/WLE

ANNEXURE IV – LIST OF VARIABLES

ANNEXURE V - DATASHEET